

68730 AND 69112, PROTEIN KINASE MOLECULES AND USES THEREFOR

Cross Reference to Related Application

[0001] This application claims the benefit of U.S. Provisional Application Number 60/258222, filed December 22, 2000, the contents of which are incorporated herein by this reference.

Background of the Invention

[0002] Phosphate tightly associated with a molecule, e.g., a protein, has been known since the late nineteenth century. Since then, a variety of covalent linkages of phosphate to proteins have been found. The most common involve esterification of phosphate to serine, threonine, and tyrosine with smaller amounts being linked to lysine, arginine, histidine, aspartic acid, glutamic acid, and cysteine. The occurrence of phosphorylated molecules, e.g., proteins, implies the existence of one or more kinases, e.g., protein kinases, capable of phosphorylating various molecules, e.g., amino acid residues on proteins, and also of phosphatases, e.g., protein phosphatases, capable of hydrolyzing various phosphorylated molecules, e.g., phosphorylated amino acid residues on proteins.

[0003] Protein kinases play critical roles in the regulation of biochemical and morphological changes associated with cellular growth and division (D'Urso *et al.* (1990) *Science* 250:786-791; Birchmeier *et al.* (1993) *Bioessays* 15:185-189). For example, these kinases have been shown to participate in the transmission of signals from growth-factor receptors (Sturgill *et al.* (1988) *Nature* 344:715-718; Gomez *et al.* (1991) *Nature* 353:170-173), control of entry of cells into mitosis (Nurse (1990) *Nature* 344:503-508; Maller (1991) *Curr. Opin. Cell Biol.* 3:269-275), and regulation of actin bundling (Husain-Chishti *et al.* (1988) *Nature* 334:718-721). Protein kinases serve as growth factor receptors and signal transducers and have been implicated in cellular transformation and malignancy (Hunter *et al.* (1992) *Cell* 70:375-387; Posada *et al.* (1992) *Mol. Biol. Cell* 3:583-592; Hunter *et al.* (1994) *Cell* 79:573-582). Alterations in kinase genes and their products can lead to deregulated cell proliferation, a hallmark of cancer. Modulation of these genes and their regulatory activities may permit the control of tumor cell proliferation and invasion.

[0004] Protein kinases can be divided into different groups based on either amino acid sequence similarity or specificity for either serine/threonine or tyrosine residues. A small

number of dual-specificity kinases have also been described. Within the broad classification, kinases can be further subdivided into families whose members share a higher degree of catalytic domain amino acid sequence identity and also have similar biochemical properties. Most protein kinase family members also share structural features outside the kinase domain that reflect their particular cellular roles. These include regulatory domains that control kinase activity or interaction with other proteins (Hanks *et al.* (1988) *Science* 241:42-52).

[0005] Extracellular signal-regulated kinases/mitogen-activated protein kinases (ERKs\MAPKs) and cyclin-directed kinases (Cdks) represent two large families of serine-threonine kinases (see Songyang *et al.* (1996) *Mol. Cell. Biol.* 16: 6486-6493). Both types of kinases function in cell growth, cell division, and cell differentiation in response to extracellular stimuli. The ERK\MAPK family members are critical participants in intracellular signaling pathways. Upstream activators as well as the ERK\MAPK components are phosphorylated following contact of cells with growth factors or hormones or in response to cellular stressors, for example, heat, ultraviolet light, and inflammatory cytokines. These kinases transport messages that have been relayed from the plasma membrane to the cytoplasm by upstream kinases into the nucleus where they phosphorylate transcription factors and effect gene transcription modulation (Karin *et al.* (1995) *Curr. Biol.* 5: 747-757). Substrates of the ERK\MAPK family include c-fos, c-jun, APF2, and ETS family members Elk1, Sap1a, and c-Ets-1 (cited in Brott *et al.* (1998) *Proc. Natl. Acad. Sci. USA* 95: 963-968).

[0006] Signal-transduction pathways that employ members of the ERK/MAPK family of serine/threonine kinases are widely conserved among eukaryotes. The multiplicity of these pathways allows the cell to respond to divergent extracellular stimuli by initiating a broad array of responses ranging from cell growth to apoptosis. ERK/MAPK pathways are comprised of a three-tiered core-signaling module wherein ERK/MAPKs are regulated by MAPK/ERK kinases (MEKs), and MEKs, in turn, are regulated by MAPK kinase kinases (MAPKKKs). Mammalian stress-activated ERK/MAPK pathways have been implicated in numerous important physiological functions, including cell growth and proliferation, inflammatory responses, and apoptosis. For example, activation of the ERK1,2 signaling pathway by a mitogenic growth factor, a tumor promoter, or by transformation suppresses decorin gene expression in fibroblasts, which in turn may

promote proliferation and migration of normal and malignant cells (Laine *et al.* (2000) *Biochem. J.* 349: 19-25).

[0007] Cdk regulate transitions between successive stages of the cell cycle. The activity of these molecules is controlled by phosphorylation events and by association with cyclin. Cdk activity is negatively regulated by the association of small inhibitory molecules (Dymlacht (1997) *Nature* 389:148-152). Cdk targets include various transcriptional activators such as p110Rb, p107, and transcription factors, such as p53, E2F, and RNA polymerase II, as well as various cytoskeletal proteins and cytoplasmic signaling proteins (cited in Brott *et al.* (1998) *Proc. Natl. Acad. Sci. USA* 95: 963-968).

[0008] Protein kinases play critical roles in cellular growth, particularly in the transduction of signals for cell proliferation, differentiation, and apoptosis. Therefore, novel protein kinase polynucleotides and proteins are useful for modulating cellular growth, differentiation, and/or development.

Summary of the Invention

[0009] The present invention is based, in part, on the discovery of two novel human protein kinases, referred to herein as "68730" and "69112". The nucleotide sequence of a cDNA encoding 68730 is shown in SEQ ID NO:1, and the amino acid sequence of a 68730 polypeptide is shown in SEQ ID NO:2. In addition, the nucleotide sequence of the coding region is depicted in SEQ ID NO:3. The nucleotide sequence of a cDNA encoding 69112 is shown in SEQ ID NO:4, and the amino acid sequence of a 69112 polypeptide is shown in SEQ ID NO:5. In addition, the nucleotide sequence of the coding region is depicted in SEQ ID NO:6.

[0010] Accordingly, in one aspect, the invention features a nucleic acid molecule which encodes a 68730 or 69112 protein or polypeptide, e.g., a biologically active portion of the 68730 or 69112 protein. In a preferred embodiment, the isolated nucleic acid molecule encodes a polypeptide having the amino acid sequence of SEQ ID NO:2 or 5. In other embodiments, the invention provides an isolated 68730 or 69112 nucleic acid molecule having the nucleotide sequence shown in SEQ ID NO:1, 3, 4, or 6. In still other embodiments, the invention provides nucleic acid molecules that are sufficiently identical (e.g., naturally occurring allelic variants) to the nucleotide sequence shown in SEQ ID NO:1, 3, 4, or 6. In other embodiments, the invention provides a nucleic acid molecule which hybridizes under stringent hybridization conditions to a nucleic acid molecule

comprising the nucleotide sequence of SEQ ID NO:1, 3, 4, or 6, wherein the nucleic acid encodes a full length 68730 or 69112 protein, or a biologically active fragment thereof.

[0011] In a related aspect, the invention further provides nucleic acid constructs which include a 68730 or 69112 nucleic acid molecule described herein. In certain embodiments, the nucleic acid molecules of the invention are operatively linked to native or heterologous regulatory sequences. Also included are vectors and host cells containing 68730 or 69112 nucleic acid molecules of the invention e.g., vectors and host cells suitable for producing 68730 or 69112 nucleic acid molecules and polypeptides.

[0012] In another related aspect, the invention provides nucleic acid fragments suitable as primers or hybridization probes for the detection of 68730- or 69112-encoding nucleic acids.

[0013] In still another related aspect, isolated nucleic acid molecules that are antisense to a 68730 or 69112 encoding nucleic acid molecule are provided.

[0014] In another aspect, the invention features 68730 or 69112 polypeptides, and biologically active or antigenic fragments thereof, that are useful, e.g., as reagents or targets in assays applicable to treatment and diagnosis of 68730- or 69112-mediated or related disorders. In another embodiment, the invention provides 68730 and 69112 polypeptides having a 68730 or 69112 activity. Preferred polypeptides are 68730 and 69112 proteins including at least one protein kinase family domain, and, preferably, having a 68730 or 69112 activity, e.g., a 68730 or 69112 activity as described herein.

[0015] In other embodiments, the invention provides 68730 and 69112 polypeptides, e.g., a 68730 polypeptide having the amino acid sequence shown in SEQ ID NO:2; a 69112 polypeptide having the amino acid sequence shown in SEQ ID NO:5; an amino acid sequence that is sufficiently identical to the amino acid sequence shown in SEQ ID NO:2 or 5; or an amino acid sequence encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, 3, 4, or 6, wherein the nucleic acid encodes a full length 68730 or 69112 protein, or a biologically active fragment thereof.

[0016] In a related aspect, the invention further provides nucleic acid constructs which include 68730 and 69112 nucleic acid molecules described herein.

[0017] In a related aspect, the invention provides 68730 and 69112 polypeptides or fragments operatively linked to non-68730 and non-69112 polypeptides to form fusion proteins.

[0018] In another aspect, the invention features antibodies and antigen-binding fragments thereof, that react with, or more preferably, specifically bind 68730 or 69112 polypeptides.

[0019] In another aspect, the invention provides methods of screening for compounds that modulate the expression or activity of the 68730 or 69112 polypeptides or nucleic acids.

[0020] In still another aspect, the invention provides a process for modulating 68730 or 69112 polypeptide or nucleic acid expression or activity, e.g. using the screened compounds. In certain embodiments, the methods involve treatment of conditions related to aberrant activity or expression of the 68730 or 69112 polypeptides or nucleic acids, such as conditions involving aberrant or deficient cellular proliferation or differentiation.

[0021] The invention also provides assays for determining the activity of or the presence or absence of 68730 or 69112 polypeptides or nucleic acid molecules in a biological sample, including for disease diagnosis.

[0022] In further aspect the invention provides assays for determining the presence or absence of a genetic alteration in a 68730 or 69112 polypeptide or nucleic acid molecule in a biological sample (using, for example, 68730 or 69112 nucleic acid molecules or anti-68730 or anti-69112 antibodies), including for disease diagnosis.

[0023] Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

Brief Description of the Drawings

[0024] *Figure 1* is a hydropathy plot of human 68730 protein in which relative hydrophobic residues are above the dashed horizontal line, and relative hydrophilic residues below the dashed horizontal line. The numbers below the plot correspond to the amino acids of the human 68730 protein sequence.

[0025] *Figure 2* is a hydropathy plot of human 69112 protein in which relative hydrophobic residues are above the dashed horizontal line, and relative hydrophilic

residues below the dashed horizontal line. The numbers below the plot correspond to the amino acids of the human 69112 protein sequence.

[0026] *Figures 3A, 3B and 3C* depict alignments of regions of the 68730 protein to the top-scoring domains identified by searching for complete domains in PFAM (version 5.5) and SMART (version 3.1) against the HMM database (HMMER 2.1.1) Fig. 5A depicts an alignment of amino acid residues 23 to 279 of SEQ ID NO:2 with a protein kinase domain (PFAM Accession No. PF00069). A single domain was found, with a bit score of 346.3 and an E-value of $3.4\text{e-}100$. Fig. 5B depicts an alignment of amino acid residues 23 to 279 of SEQ ID NO:2 with a serine/threonine kinase domain (SMART Accession No. S_TKc). The bit score is 386.6 and the E-value is $2.5\text{e-}112$. Fig. 5C depicts an alignment of amino acid residues 23 to 279 of SEQ ID NO:2 with a tyrosine kinase domain (SMART Accession No. TyrKc). The bit score is 35.7 and the E-value is $4\text{e-}14$. All three alignments show four underlined sequences. The first underlined sequence (at amino acid residues 29 to 57) is a pattern match to a protein kinase ATP binding region signature sequence (Prosite Accession No. PS00107). The second and third underlined sequences (at amino acid residues 109 to 116 and at amino acids 126 to 134) are pattern matches to Prosite Accession No. PS00007, a tyrosine kinase phosphorylation site. The fourth underlined sequence (at amino acid residues 140 to 152) is a pattern match to a serine/threonine protein kinase active site signature sequence (Prosite Accession No. PS00108).

[0027] *Figures 4A, 4B and 4C* depict alignments of the 69112 protein to the top-scoring domains identified by searching for complete domains in PFAM (version 5.5) and SMART (version 3.1) against the HMM database (HMMER 2.1.1) Fig. 6A depicts an alignment of amino acid residues 356 to 613 of SEQ ID NO:5 with a protein kinase domain (PFAM Accession No. PF00069). A single domain was found, with a bit score of 350.2 and an E-value of $2.3\text{e-}101$. Fig. 6B depicts an alignment of amino acid residues 356 to 613 of SEQ ID NO:5 with a serine/threonine kinase domain (SMART Accession No. S_TKc). The bit score is 372.7 and the E-value is $3.8\text{e-}108$. Fig. 6C depicts an alignment of amino acid residues 356 to 613 of SEQ ID NO:5 with a tyrosine kinase domain (SMART Accession No. TyrKc). The bit score is 50.0 and the E-value is $5.3\text{e-}15$. All three alignments show an underlined sequence at 473 to 485, which is a pattern match to a serine/threonine protein kinase active site signature sequence (Prosite Accession No. PS00108).

Detailed Description

[0028] The human 68730 sequence (SEQ ID NO:1), which is approximately 1772 nucleotides long including untranslated regions, contains a predicted methionine-initiated coding sequence of about 1074 nucleotides (nucleotides 169 to 1242 of SEQ ID NO:1; nucleotides 1 to 1074 of SEQ ID NO:3), including the termination codon. The coding sequence encodes a 357 amino acid protein (SEQ ID NO:2).

[0029] The human 69112 sequence (SEQ ID NO:4), which is approximately 3579 nucleotides long including untranslated regions, contains a predicted methionine-initiated coding sequence of about 1947 nucleotides (nucleotides 1213 to 3159 of SEQ ID NO:4; nucleotides 1 to 1947 of SEQ ID NO:6), including the termination codon. The coding sequence encodes a 648 amino acid protein (SEQ ID NO:5).

[0030] To determine whether a polypeptide or protein of interest has a conserved sequence or domain common to members of a protein family, the amino acid sequence of the protein can be searched against a database of HMMs (e.g., the Pfam database, release 2.1) using the default parameters

(http://www.sanger.ac.uk/Software/Pfam/HMM_search). For example, the hmmsf program, which is available as part of the HMMER package of search programs, is a family specific default program for MILPAT0063 and a score of 15 is the default threshold score for determining a hit. Alternatively, the threshold score for determining a hit can be lowered (e.g., to 8 bits). A description of the Pfam database can be found in Sonnhammer et al., (1997) *Proteins* 28(3):405-420 and a detailed description of HMMs can be found, for example, in Gribskov et al., (1990) *Meth. Enzymol.* 183:146-159; Gribskov et al., (1987) *Proc. Natl. Acad. Sci. USA* 84:4355-4358; Krogh et al., (1994) *J. Mol. Biol.* 235:1501-1531; and Stultz et al., (1993) *Protein Sci.* 2:305-314, the contents of which are incorporated herein by reference. See also, for example, *The HMMER User's Guide* at <http://hmmer.wustl.edu/hmmer.html>. For general information regarding PFAM identifiers, PS prefix and PF prefix domain identification numbers, refer to Sonnhammer et al. (1997) *Protein* 28:405-420 and <http://www.psc.edu/general/software/packages/pfam/pfam.html>. See also, for example, <http://www.expasy.ch/prosite> and <http://smart.embl-heidelberg.de/>.

[0031] Using such search tools, the 68730 and 69112 protein sequences were found to contain significant structural characteristics in common with members of the protein

kinase family of molecules. Some of these structural characteristics include, for example, a protein kinase domain, such as the consensus sequence of PFAM Accession No. PF00069 (see Figures 3A and 4A, which show an alignment of amino acids 23 to 279 of SEQ ID NO:2 and amino acids 356 to 613 of SEQ ID NO:5 with PFAM Accession No. PF00069); and a serine/threonine kinase catalytic domain, such as the consensus sequence identified by SMART as Accession No. S_TKc (see Figures 3B and 4B, which show an alignment of amino acids 23 to 279 of SEQ ID NO:2 and amino acid residues 356 to 613 of SEQ ID NO:5 with SMART Accession No. S_TKc). Another example of a common structural characteristic can include, for example, a tyrosine kinase domain, such as the consensus sequence of SMART Accession No. TyrKc (see Figures 3C and 4C, which show an alignment of amino acid residues 23 to 279 of SEQ ID NO:2 and amino acid residues 356 to 613 of SEQ ID NO:5 with SMART Accession No. TyrKc).

[0032] As used herein, the term "protein kinase" includes a protein or polypeptide which is capable of modulating its own phosphorylation state or the phosphorylation state of another molecule, e.g., protein or polypeptide. Protein kinases can have a specificity for (i.e., a specificity to phosphorylate) serine/threonine residues, tyrosine residues, or both serine/threonine and tyrosine residues, e.g., the dual specificity kinases. As used herein, a "protein kinase domain" includes a consensus sequence, e.g., PFAM Accession No. PF00069, that includes the catalytic domain of the enzyme. The catalytic domain can be characterized by the presence of an ATP binding signature sequence (e.g., Prosite Accession No. PS00107) and/or a serine/threonine kinase active-site signature sequence (e.g., Prosite Accession No. PS00108). The protein kinase domain of the present invention preferably includes a catalytic domain of about 150-400 amino acid residues in length, preferably about 200-300 amino acid residues in length, or more preferably about 225-300 amino acid residues in length, which includes at least one of the signature sequences or motifs described herein.

[0033] The term "family" when referring to the protein and nucleic acid molecules of the invention is intended to mean two or more proteins or nucleic acid molecules having a common structural domain or signature sequence and having sufficient amino acid or nucleotide sequence homology as defined herein. Such family members can be naturally or non-naturally occurring and can be from either the same or different species. For example, a family can contain a first protein of human origin, as well as other distinct proteins of human origin, or alternatively, can contain homologues of non-human origin,

e.g., rat or mouse proteins. Members of a family can also have common functional characteristics.

[0034] With regard to common structural characteristics described above, the protein kinases of the present invention include a protein kinase catalytic core or domain and can include at least one of the following signature sequences or motifs within the catalytic core: a protein kinase ATP-binding region signature sequence, a signature sequence that confers on the kinase its specificity for phosphorylation of either tyrosine or serine/threonine residues, and a tyrosine kinase phosphorylation site (See Hanks et al. (1988) *Science* 241:42-52).

[0035] The protein kinase ATP-binding region signature sequence is located in the N-terminal extremity of the catalytic domain and typically includes a glycine-rich stretch of residues in the vicinity of a lysine residue. A consensus sequence (Prosite Accession No. PS00107; SEQ ID NO:7) for this region is [LIV]-G-{P}-G-{P}-[FYWMGSTNH]-[SGA]-{PW}-[LIVCAT]-{PD}-x-[GSTACLIVMFY]-x(5,18)-[LIVMFYWCSTAR]-[AIVP]-[LIVMFAGCKR]-K.

[0036] In this and the following consensus sequence patterns, each element in the pattern is separated by a dash (-); square [] brackets indicate the particular residues that are accepted at that position; elaborate{ } brackets indicate the residues that are not accepted at that position; x indicates any residue is accepted at that position; repetition of a particular element is indicated by following the element with a numerical value or a numerical range enclosed in parentheses (i.e., above, x(5,18) indicates anywhere from 5 to 18 residues are present in the element, and any residue can be accepted at each of these 5 to 18 residue positions); and the standard IUPAC one-letter code for the amino acids is used. In the above consensus sequence pattern, lysine (K) binds ATP.

[0037] Analysis of the 68730 polypeptide for sequence patterns in the Prosite database showed a match to a protein kinase ATP binding signature pattern (Prosite Accession No. PS00107) at about amino acids 29 to 57 (LGTGAFSEVVLAEEKATGKLFAVKCIPKK) of SEQ ID NO:2. The lysine residue at position 43, 47, 52, 56, and/or 57 can be involved in ATP binding. This protein kinase ATP-binding region signature sequence lies within a protein kinase domain spanning amino acid residues 23 to 279 of SEQ ID NO:2, as determined by a search using PFAM and SMART against the HMM database (HMMER 2.1.1) and is shown in Figures 3A-3C as the first underlined sequence.

[0038] Another region, located in the central part of the catalytic core or domain, contains a conserved aspartic acid residue, which is important for the catalytic activity of the enzyme (Knighton *et al.* (1991) *Science* 253:407-414). Two active-site signature sequences have been described for this region: one specific for serine/threonine kinases and one for tyrosine kinases. In both signature sequences aspartic acid (D) is conserved and is an active site residue. A consensus sequence for the serine/threonine kinases (Prosite Accession No. PS00108; SEQ ID NO:8) is [LIVMFYC]-x-[HY]-x-D-[LIVMFY]-K-x(2)-N-[LIVMFYCT](3).

[0039] A consensus sequence for the tyrosine kinases (Prosite Accession No. PS00109; SEQ ID NO:9) is [LIVMFYC]-x-[HY]-x-D-[LIVMFY]-[RSTAC]-x(2)-N-[LIVMFYC](3).

[0040] Analysis of the 68730 polypeptide for sequence patterns in the Prosite database showed a match of IVHRDLKPENLLY (amino acid residues 140 to 152 of SEQ ID NO:2) to the serine/threonine kinase active-site signature sequence shown above (PS00108; SEQ ID NO:8). The aspartic acid residue at position 144 of SEQ ID NO:2 is an active site residue. This serine/threonine kinase active-site signature sequence lies within a protein kinase domain spanning amino acid residues 23 to 279 of SEQ ID NO:2, as determined by a search using PFAM and SMART against the HMM database (HMMER 2.1.1) and is shown in Figures 3A-3C as the fourth underlined sequence.

[0041] Analysis of the 69112 polypeptide for sequence patterns in the Prosite database showed a match of IVHRDLKPENLLV (amino acid residues 473 to 485 of Figure 2A and SEQ ID NO:5) to the serine/threonine kinase active-site signature sequence shown above (PS00108; SEQ ID NO:8). The aspartic acid residue at position 477 of SEQ ID NO:5 is an active site residue. This serine/threonine kinase active-site signature sequence lies within a protein kinase domain spanning amino acid residues 356 to 613 of SEQ ID NO:5, as determined by a search using PFAM and SMART against the HMM database (HMMER 2.1.1) and is shown in Figures 4A-4C as the underlined sequence.

[0042] Substrates of tyrosine protein kinases are generally characterized by a lysine or an arginine seven residues to the N-terminal side of the phosphorylated tyrosine. An acidic residue (aspartic acid or glutamic acid) is often found at either three or four residues to the N-terminal side of the tyrosine (see Patschinsky *et al.* (1982) *Proc. Natl. Acad. Sci. U.S.A.* 79:973-977; Hunter T. (1982) *J. Biol. Chem.* 257:4843-4848; Cooper *et*

al. (1984) *J. Biol. Chem.* 259:7835-7841). Prosite Accession No. PS00007 is a consensus pattern for a tyrosine kinase phosphorylation site with the following sequence (SEQ ID NO:10): [RK]-x(2,3)-[DE]-x(2,3)-Y. In this sequence, tyrosine (Y) is the phosphorylation site.

[0043] Analysis of the 68730 polypeptide indicates that this sequence pattern occurs twice in the protein kinase domain spanning amino acid residues 23 to 279 of SEQ ID NO:2, shown in Figures 3A-3C as the second and third underlined sequences: in the sequence RIVEKGFY at amino acid residues 109 to 116, and in the sequence RQVLDAVYY at amino acid residues 126 to 134, wherein the phosphorylation site for these respective motifs reside at the tyrosine residue at positions 116 and 134.

[0044] The sequence analyses of the 68730 and 69112 proteins demonstrate that: 1) the 68730 protein can act as a serine/threonine protein kinase, and can also include at least one tyrosine kinase phosphorylation site, indicating that it can be phosphorylated by itself (i.e., autophosphorylation) or by another kinase; and 2) the 69112 protein is a serine/threonine protein kinase.

[0045] Isolated proteins of the present invention, preferably 68730 and 69112 proteins, have an amino acid sequence sufficiently homologous to the amino acid sequence of SEQ ID NO:2 or 5, or are encoded by a nucleotide sequence sufficiently homologous or sufficiently identical to SEQ ID NO:1, 3, 4, or 6. As used interchangeably herein, the terms "sufficiently homologous" and "sufficiently identical" refer to a first amino acid or nucleotide sequence which contains a sufficient or minimum number of identical or equivalent (e.g., an amino acid residue which has a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences share common structural domains or motifs and/or a common functional activity. For example, amino acid or nucleotide sequences which share common structural domains have at least 30%, 40%, or 50% homology, preferably 60% homology, more preferably 70%-80%, and even more preferably 90-95% homology across the amino acid sequences of the domains and contain at least one and preferably two structural domains or motifs, are defined herein as sufficiently homologous. Furthermore, amino acid or nucleotide sequences which share at least 30%, 40%, or 50%, preferably 60%, more preferably 70-80%, or 90-95% homology and share a common functional activity are defined herein as sufficiently homologous.

[0046] As used interchangeably herein, a "68730 activity", "biological activity of 68730" or "functional activity of 68730", refers to an activity exerted by a 68730 protein, polypeptide or nucleic acid molecule on a 68730 responsive cell or a 68730 protein substrate, as determined *in vivo*, or *in vitro*, according to standard techniques. As used interchangeably herein, a "69112 activity", "biological activity of 69112" or "functional activity of 69112", refers to an activity exerted by a 69112 protein, polypeptide or nucleic acid molecule on a 69112 responsive cell or a 69112 protein substrate, as determined *in vivo*, or *in vitro*, according to standard techniques.

[0047] Protein kinases play a role in signalling pathways associated with cellular growth. For example, protein kinases are involved in the regulation of signal transmission from cellular receptors, e.g., growth-factor receptors; entry of cells into mitosis; and the regulation of cytoskeleton function, e.g., actin bundling. Thus, the 68730 and 69112 molecules of the present invention can be involved in: 1) the regulation of transmission of signals from cellular receptors, e.g., cell growth factor receptors; 2) the modulation of the entry of cells, e.g., precursor cells, into mitosis; 3) the modulation of cellular differentiation; 4) the modulation of cell death; and 5) the regulation of cytoskeleton function, e.g., actin bundling. These kinases can function in these biological activities because of their ability to phosphorylate themselves or other substrate molecules.

[0048] Inhibition or over stimulation of the activity of protein kinases involved in signaling pathways associated with cellular growth can lead to perturbed cellular growth, which can in turn lead to cellular growth related disorders. As used herein, a "cellular growth related disorder" includes a disorder, disease, or condition characterized by a deregulation, e.g., an upregulation or a downregulation, of cellular growth. Cellular growth deregulation may be due to a deregulation of cellular proliferation, cell cycle progression, cellular differentiation and/or cellular hypertrophy. Examples of cellular growth related disorders include cardiovascular disorders such as heart failure, hypertension, atrial fibrillation, dilated cardiomyopathy, idiopathic cardiomyopathy, or angina; proliferative disorders or differentiative disorders such as cancer, e.g., melanoma, prostate cancer, cervical cancer, breast cancer, colon cancer, or sarcoma.

[0049] Accordingly, another embodiment of the invention features isolated 68730 and 69112 proteins and polypeptides having a 68730 or 69112 activity. Preferred proteins are 68730 and 69112 proteins having at least one protein kinase domain, at least a protein

kinase ATP-binding signature sequence or a serine/threonine kinase active-site signature sequence, and, preferably, a 68730 or 69112 activity. Additional preferred 68730 and 69112 proteins have at least one protein kinase domain, at least one of a protein kinase ATP-binding signature sequence or a serine/threonine kinase active-site signature sequence, and are, preferably, encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, 3, 4, or 6, or a complement thereof.

[0050] The 68730 and 69112 polypeptides exhibit a domain shared by protein kinases and thus have similar biological activities. Accordingly, 68730 and 69112 can play a role (*e.g.*, involving a protein kinase domain) in cell proliferation and cancer, inflammation and apoptosis, and thus the 68730 and 69112 compositions of the invention (*e.g.*, nucleic acids, polypeptides, proteins, antibodies, and small molecule modulators of 68730 and 69112) can be used to modulate cell proliferation, *e.g.*, in cancer, inflammation or apoptosis, and furthermore can be used in screening assays to identify agents for modulating cell proliferation, as well as in detection or diagnostic assays to identify conditions involving aberrant cell proliferation.

[0051] As used herein, a "protein kinase-associated disorder" includes a disorder, disease or condition which is caused by, characterized by, or associated with a misregulation (*e.g.*, downregulation or upregulation) of a protein kinase-mediated activity or an abnormal protein kinase-mediated activity. Protein kinase-associated disorders can detrimentally affect cellular functions such as cellular proliferation, growth, differentiation, or migration, cellular regulation of homeostasis, inter- or intra-cellular communication; tissue function, such as cardiac function or musculoskeletal function; systemic responses in an organism, such as nervous system responses, hormonal responses (*e.g.*, insulin response), or immune responses; and protection of cells from toxic compounds (*e.g.*, carcinogens, toxins, mutagens, and toxic byproducts of metabolic activity (*e.g.*, reactive oxygen species)). Accordingly, the 68730 and 69112 molecules of the invention, as protein kinases, can mediate various protein kinase-associated disorders, including cellular proliferative and/or differentiative disorders, hormonal disorders, immune and inflammatory disorders, neurological disorders, cardiovascular disorders, blood vessel disorders, and platelet disorders. As the 68730 and 69112 molecules of the invention can modulate protein kinase-mediated activities, they are useful for developing

novel diagnostic and therapeutic agents for 68730- or 69112-mediated or related disorders, as described below.

[0052] Examples of cellular proliferative and/or differentiative disorders include cancer, e.g., carcinoma, sarcoma, metastatic disorders or hematopoietic neoplastic disorders, e.g., leukemias. A metastatic tumor can arise from a multitude of primary tumor types, including but not limited to those of prostate, colon, lung, breast and liver origin.

[0053] As used herein, the term "cancer" (also used interchangeably with the terms, "hyperproliferative" and "neoplastic") refers to cells having the capacity for autonomous growth, i.e., an abnormal state or condition characterized by rapidly proliferating cell growth. Cancerous disease states may be categorized as pathologic, i.e., characterizing or constituting a disease state, e.g., malignant tumor growth, or may be categorized as non-pathologic, i.e., a deviation from normal but not associated with a disease state, e.g., cell proliferation associated with wound repair. The term is meant to include all types of cancerous growths or oncogenic processes, metastatic tissues or malignantly transformed cells, tissues, or organs, irrespective of histopathologic type or stage of invasiveness. The term "cancer" includes malignancies of the various organ systems, such as those affecting lung, breast, thyroid, lymphoid, gastrointestinal, and genito-urinary tract, as well as adenocarcinomas which include malignancies such as most colon cancers, renal-cell carcinoma, prostate cancer and/or testicular tumors, non-small cell carcinoma of the lung, cancer of the small intestine and cancer of the esophagus. The term "carcinoma" is art recognized and refers to malignancies of epithelial or endocrine tissues including respiratory system carcinomas, gastrointestinal system carcinomas, genitourinary system carcinomas, testicular carcinomas, breast carcinomas, prostatic carcinomas, endocrine system carcinomas, and melanomas. Exemplary carcinomas include those forming from tissue of the cervix, lung, prostate, breast, head and neck, colon and ovary. The term "carcinoma" also includes carcinosarcomas, e.g., which include malignant tumors composed of carcinomatous and sarcomatous tissues. An "adenocarcinoma" refers to a carcinoma derived from glandular tissue or in which the tumor cells form recognizable glandular structures. The term "sarcoma" is art recognized and refers to malignant tumors of mesenchymal derivation.

[0054] The 68730 and 69112 molecules of the invention can be used to monitor, treat and/or diagnose a variety of proliferative disorders. Such disorders include hematopoietic

neoplastic disorders. As used herein, the term "hematopoietic neoplastic disorders" includes diseases involving hyperplastic/neoplastic cells of hematopoietic origin, *e.g.*, arising from myeloid, lymphoid or erythroid lineages, or precursor cells thereof. Typically, the diseases arise from poorly differentiated acute leukemias, *e.g.*, erythroblastic leukemia and acute megakaryoblastic leukemia. Additional exemplary myeloid disorders include, but are not limited to, acute promyeloid leukemia (APML), acute myelogenous leukemia (AML) and chronic myelogenous leukemia (CML) (reviewed in Vaickus, L., (1991) *Crit. Rev. in Oncol./Hematol.* 11:267-97); lymphoid malignancies include, but are not limited to acute lymphoblastic leukemia (ALL) which includes B-lineage ALL and T-lineage ALL, chronic lymphocytic leukemia (CLL), prolymphocytic leukemia (PLL), hairy cell leukemia (HLL) and Waldenstrom's macroglobulinemia (WM). Additional forms of malignant lymphomas include, but are not limited to non-Hodgkin lymphoma and variants thereof, peripheral T cell lymphomas, adult T cell leukemia/lymphoma (ATL), cutaneous T-cell lymphoma (CTCL), large granular lymphocytic leukemia (LGF), Hodgkin's disease and Reed-Sternberg disease.

[0055] Protein kinase-associated disorders can include hormonal disorders, such as conditions or diseases in which the production and/or regulation of hormones in an organism is aberrant. Examples of such disorders and diseases include type I and type II diabetes mellitus, pituitary disorders (*e.g.*, growth disorders), thyroid disorders (*e.g.*, hypothyroidism or hyperthyroidism), and reproductive or fertility disorders (*e.g.*, disorders which affect the organs of the reproductive system, *e.g.*, the prostate gland, the uterus, or the vagina; disorders which involve an imbalance in the levels of a reproductive hormone in a subject; disorders affecting the ability of a subject to reproduce; and disorders affecting secondary sex characteristic development, *e.g.*, adrenal hyperplasia).

[0056] Protein kinase-associated disorders also include immune disorders, such as autoimmune disorders or immune deficiency disorders, *e.g.*, congenital X-linked infantile hypogammaglobulinemia, transient hypogammaglobulinemia, common variable immunodeficiency, selective IgA deficiency, chronic mucocutaneous candidiasis, or severe combined immunodeficiency. Other examples of disorders include autoimmune diseases (including, for example, diabetes mellitus, arthritis (including rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis, psoriatic arthritis), multiple sclerosis, encephalomyelitis, myasthenia gravis, systemic lupus erythematosus, autoimmune thyroiditis, dermatitis (including atopic dermatitis and eczematous

dermatitis), psoriasis, Sjögren's Syndrome, inflammatory bowel disease (e.g., Crohn's disease and ulcerative colitis), aphthous ulcer, iritis, conjunctivitis, keratoconjunctivitis, respiratory inflammation (e.g., asthma, allergic asthma, and chronic obstructive pulmonary disease), cutaneous lupus erythematosus, scleroderma, vaginitis, proctitis, drug eruptions, leprosy reversal reactions, erythema nodosum leprosum, autoimmune uveitis, allergic encephalomyelitis, acute necrotizing hemorrhagic encephalopathy, idiopathic bilateral progressive sensorineural hearing loss, aplastic anemia, pure red cell anemia, idiopathic thrombocytopenia, polychondritis, Wegener's granulomatosis, chronic active hepatitis, Stevens-Johnson syndrome, idiopathic sprue, lichen planus, Graves' disease, sarcoidosis, primary biliary cirrhosis, uveitis posterior, and interstitial lung fibrosis), graft-versus-host disease, cases of transplantation, and allergy such as, atopic allergy.

[0057] Additional protein kinase-associated disorders are neurological disorders. Such neurological disorders include, for example, disorders involving neurons, and disorders involving glia, such as astrocytes, oligodendrocytes, ependymal cells, and microglia; cerebral edema, raised intracranial pressure and herniation, and hydrocephalus; malformations and developmental diseases, such as neural tube defects, forebrain anomalies, posterior fossa anomalies, and syringomyelia and hydromyelia; perinatal brain injury; cerebrovascular diseases, such as those related to hypoxia, ischemia, and infarction, including hypotension, hypoperfusion, and low-flow states--global cerebral ischemia and focal cerebral ischemia--infarction from obstruction of local blood supply, intracranial hemorrhage, including intracerebral (intraparenchymal) hemorrhage, subarachnoid hemorrhage and ruptured berry aneurysms, and vascular malformations, hypertensive cerebrovascular disease, including lacunar infarcts, slit hemorrhages, and hypertensive encephalopathy; infections, such as acute meningitis, including acute pyogenic (bacterial) meningitis and acute aseptic (viral) meningitis, acute focal suppurative infections, including brain abscess, subdural empyema, and extradural abscess, chronic bacterial meningoenitis, including tuberculosis and mycobacterioses, neurosyphilis, and neuroborreliosis (Lyme disease), viral meningoenitis, including arthropod-borne (Arbo) viral encephalitis, *Herpes simplex* virus Type 1, *Herpes simplex* virus Type 2, *Varicella-zoster* virus (*Herpes zoster*), cytomegalovirus, poliomyelitis, rabies, and human immunodeficiency virus 1, including HIV-1 meningoenitis (subacute encephalitis), vacuolar myelopathy, AIDS-

associated myopathy, peripheral neuropathy, and AIDS in children, progressive multifocal leukoencephalopathy, subacute sclerosing panencephalitis, fungal meningoencephalitis, other infectious diseases of the nervous system; transmissible spongiform encephalopathies (prion diseases); demyelinating diseases, including multiple sclerosis, multiple sclerosis variants, acute disseminated encephalomyelitis and acute necrotizing hemorrhagic encephalomyelitis, and other diseases with demyelination; degenerative diseases, such as degenerative diseases affecting the cerebral cortex, including Alzheimer's disease and Pick's disease, degenerative diseases of basal ganglia and brain stem, including Parkinsonism, idiopathic Parkinson disease (paralysis agitans), progressive supranuclear palsy, corticobasal degeneration, multiple system atrophy, including striatonigral degeneration, Shy-Drager syndrome, and olivopontocerebellar atrophy, and Huntington's disease; spinocerebellar degenerations, including spinocerebellar ataxias, including Friedreich ataxia, and ataxia-telangiectasia, degenerative diseases affecting motor neurons, including amyotrophic lateral sclerosis (motor neuron disease), bulbospinal atrophy (Kennedy syndrome), and spinal muscular atrophy; inborn errors of metabolism, such as leukodystrophies, including Krabbe disease, metachromatic leukodystrophy, adrenoleukodystrophy, Pelizaeus-Merzbacher disease, and Canavan disease, mitochondrial encephalomyopathies, including Leigh disease and other mitochondrial encephalomyopathies; toxic and acquired metabolic diseases, including vitamin deficiencies such as thiamine (vitamin B₁) deficiency and vitamin B₁₂ deficiency, neurologic sequelae of metabolic disturbances, including hypoglycemia, hyperglycemia, and hepatic encephatopathy, toxic disorders, including carbon monoxide, methanol, ethanol, and radiation, including combined methotrexate and radiation-induced injury; tumors, such as gliomas, including astrocytoma, including fibrillary (diffuse) astrocytoma and glioblastoma multiforme, pilocytic astrocytoma, pleomorphic xanthoastrocytoma, and brain stem glioma, oligodendroglioma, and ependymoma and related paraventricular mass lesions, neuronal tumors, poorly differentiated neoplasms, including medulloblastoma, other parenchymal tumors, including primary brain lymphoma, germ cell tumors, and pineal parenchymal tumors, meningiomas, metastatic tumors, paraneoplastic syndromes, peripheral nerve sheath tumors, including schwannoma, neurofibroma, and malignant peripheral nerve sheath tumor (malignant schwannoma), and neurocutaneous syndromes (phakomatoses),

including neurofibromatosis, including Type 1 neurofibromatosis (NF1) and TYPE 2 neurofibromatosis (NF2), tuberous sclerosis, and Von Hippel-Lindau disease.

[0058] Cardiovascular disorders include, but are not limited to, heart failure, including but not limited to, cardiac hypertrophy, left-sided heart failure, and right-sided heart failure; ischemic heart disease, including but not limited to angina pectoris, myocardial infarction, chronic ischemic heart disease, and sudden cardiac death; hypertensive heart disease, including but not limited to, systemic (left-sided) hypertensive heart disease and pulmonary (right-sided) hypertensive heart disease; valvular heart disease, including but not limited to, valvular degeneration caused by calcification, such as calcification of a congenitally bicuspid aortic valve, and mitral annular calcification, and myxomatous degeneration of the mitral valve (mitral valve prolapse), rheumatic fever and rheumatic heart disease, infective endocarditis, and noninfected vegetations, such as nonbacterial thrombotic endocarditis and endocarditis of systemic lupus erythematosus (Libman-Sacks disease), carcinoid heart disease, and complications of artificial valves; myocardial disease, including but not limited to dilated cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy, and myocarditis; pericardial disease, including but not limited to, pericardial effusion and hemopericardium and pericarditis, including acute pericarditis and healed pericarditis, and rheumatoid heart disease; neoplastic heart disease, including but not limited to, primary cardiac tumors, such as myxoma, lipoma, papillary fibroelastoma, rhabdomyoma, and sarcoma, and cardiac effects of noncardiac neoplasms; congenital heart disease, including but not limited to, left-to-right shunts--late cyanosis, such as atrial septal defect, ventricular septal defect, patent ductus arteriosus, and atrioventricular septal defect, right-to-left shunts--early cyanosis, such as tetralogy of fallot, transposition of great arteries, truncus arteriosus, tricuspid atresia, and total anomalous pulmonary venous connection, obstructive congenital anomalies, such as coarctation of aorta, pulmonary stenosis and atresia, and aortic stenosis and atresia, disorders involving cardiac transplantation, and congestive heart failure.

[0059] Blood platelet disorders include, but are not limited to, thrombocytopenia due to a reduced number of megakaryocytes in the bone marrow, for example, as a result of chemotherapy; invasive disorders, such as leukemia, idiopathic or drug- or toxin-induced aplasia of the marrow, or rare hereditary amegakaryocytic thrombocytopenias; ineffective thrombopoiesis, for example, as a result of megaloblastic anemia, alcohol toxicity,

vitamin B12 or folate deficiency, myelodysplastic disorders, or rare hereditary disorders (e.g., Wiskott-Aldrich syndrome and May-hegglin anomaly); a reduction in platelet distribution, for example, as a result of cirrhosis, a splenic invasive disease (e.g., Gaucher's disease), or myelofibrosis with extramedullary myeloid metaplasia; increased platelet destruction, for example, as a result of removal of IgG-coated platelets by the mononuclear phagocytic system (e.g., idiopathic thrombocytopenic purpura (ITP), secondary immune thrombocytopenia (e.g., systemic lupus erythematosus, lymphoma, or chronic lymphocytic leukemia), drug-related immune thrombocytopenias (e.g., as with quinidine, aspirin, and heparin), post-transfusion purpura, and neonatal thrombocytopenia as a result of maternal platelet autoantibodies or maternal platelet alloantibodies). Also included are thrombocytopenia secondary to intravascular clotting and thrombin induced damage to platelets as a result of, for example, obstetric complications, metastatic tumors, severe gram-negative bacteremia, thrombotic thrombocytopenic purpura, or severe illness. Also included is dilutional thrombocytopenia, for example, due to massive hemorrhage. Blood platelet disorders also include, but are not limited to, essential thrombocytosis and thrombocytosis associated with, for example, splenectomy, acute or chronic inflammatory diseases, hemolytic anemia, carcinoma, Hodgkin's disease, lymphoproliferative disorders, and malignant lymphomas.

[0060] Disorders involving blood vessels include, but are not limited to, responses of vascular cell walls to injury, such as endothelial dysfunction and endothelial activation and intimal thickening; vascular diseases including, but not limited to, congenital anomalies, such as arteriovenous fistula, atherosclerosis, and hypertensive vascular disease, such as hypertension; inflammatory disease--the vasculitides, such as giant cell (temporal) arteritis, Takayasu arteritis, polyarteritis nodosa (classic), Kawasaki syndrome (mucocutaneous lymph node syndrome), microscopic polyanglitis (microscopic polyarteritis, hypersensitivity or leukocytoclastic angitis), Wegener granulomatosis, thromboanglitis obliterans (Buerger disease), vasculitis associated with other disorders, and infectious arteritis; Raynaud disease; aneurysms and dissection, such as abdominal aortic aneurysms, syphilitic (luetetic) aneurysms, and aortic dissection (dissecting hematoma); disorders of veins and lymphatics, such as varicose veins, thrombophlebitis and phlebothrombosis, obstruction of superior vena cava (superior vena cava syndrome), obstruction of inferior vena cava (inferior vena cava syndrome), and lymphangitis and lymphedema; tumors, including benign tumors and tumor-like conditions, such as

hemangioma, lymphangioma, glomus tumor (glomangioma), vascular ectasias, and bacillary angiomatosis, and intermediate-grade (borderline low-grade malignant) tumors, such as Kaposi's sarcoma and hemangioendothelioma, and malignant tumors, such as angiosarcoma and hemangiopericytoma; and pathology of therapeutic interventions in vascular disease, such as balloon angioplasty and related techniques and vascular replacement, such as coronary artery bypass graft surgery.

[0061] The 68730 and 69112 proteins, fragments thereof, and derivatives and other variants of the sequence in SEQ ID NO:2 and 5, are collectively referred to as "polypeptides or proteins of the invention" or "68730 and 69112 polypeptides or proteins". Nucleic acid molecules encoding such polypeptides or proteins are collectively referred to as "nucleic acids of the invention" or "68730 and 69112 nucleic acids." 68730 molecules refer to 68730 nucleic acids, polypeptides, and antibodies, and 69112 molecules refer to 69112 nucleic acids, polypeptides, and antibodies.

[0062] As used herein, the term "nucleic acid molecule" includes DNA molecules (e.g., a cDNA or genomic DNA) and RNA molecules (e.g., an mRNA) and analogs of the DNA or RNA generated, e.g., by the use of nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

[0063] The term "isolated or purified nucleic acid molecule" includes nucleic acid molecules which are separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. For example, with regards to genomic DNA, the term "isolated" includes nucleic acid molecules which are separated from the chromosome with which the genomic DNA is naturally associated. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (i.e., sequences located at the 5' and/or 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated nucleic acid molecule can contain less than about 5 kb, 4kb, 3kb, 2kb, 1 kb, 0.5 kb or 0.1 kb of 5' and/or 3' nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be sufficiently free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized.

[0064] As used herein, the term "hybridizes under stringent conditions" describes conditions for hybridization and washing. Stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. Aqueous and nonaqueous methods are described in that reference and either can be used. A preferred example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 50°C. Another example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 55°C. A further example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 60°C. Another preferred stringent hybridization condition is hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 65°C. Particularly preferred stringency conditions (and the conditions that should be used if the practitioner is uncertain about what conditions should be applied to determine if a molecule is within a hybridization limitation of the invention) are 0.5M Sodium Phosphate, 7% SDS at 65°C, followed by one or more washes at 0.2X SSC, 1% SDS at 65°C. Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO:1, or SEQ ID NO:3, corresponds to a naturally-occurring nucleic acid molecule.

[0065] As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (e.g., encodes a natural protein).

[0066] As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules which include an open reading frame encoding a 68730 or 69112 protein, preferably a mammalian 68730 or 69112 protein, and can further include non-coding regulatory sequences, and introns.

[0067] An "isolated" or "purified" polypeptide or protein is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. In one embodiment, the language "substantially free" means a preparation of 68730 or 69112 protein having less than about 30%, 20%, 10%

and more preferably 5% (by dry weight), of non-68730 or non-69112 protein (also referred to herein as a "contaminating protein"), or of chemical precursors or non-68730 or non-69112 chemicals. When the 68730 or 69112 protein, or biologically active portion thereof, is recombinantly produced, it is also preferably substantially free of culture medium, i.e., culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation. The invention includes isolated or purified preparations of at least 0.01, 0.1, 1.0, and 10 milligrams in dry weight.

[0068] A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of 68730 or 69112 (e.g., the sequence of SEQ ID NO:1, 3, 4, or 6) without abolishing or more preferably, without substantially altering a biological activity, whereas an "essential" amino acid residue results in such a change. For example, amino acid residues that are conserved among the polypeptides of the present invention, e.g., those present in the protein kinase domain, are predicted to be particularly unamenable to alteration.

[0069] A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in a 68730 or 69112 protein is preferably replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a 68730 or 69112 coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for 68730 or 69112 biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NO:1, 3, 4, or 6, the encoded protein can be expressed recombinantly and the activity of the protein can be determined.

[0070] Biologically active portions of a 68730 or 69112 protein include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino

acid sequence of the 68730 or 69112 protein, e.g., the amino acid sequence shown in SEQ ID NO:2 or 5, which include less amino acids than the full length 68730 or 69112 proteins, and exhibit at least one activity of a 68730 or 69112 protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the 68730 or 69112 protein, e.g., protein kinase activity. A biologically active portion of a 68730 or 69112 protein can be a polypeptide which is, for example, 10, 25, 50, 100, 200 or more amino acids in length. Biologically active portions of a 68730 or 69112 protein can be used as targets for developing agents which modulate a 68730 or 69112 mediated activity, e.g., protein kinase activity.

[0071] Calculations of homology or sequence identity between sequences (the terms are used interchangeably herein) are performed as follows.

[0072] To determine the percent identity of two amino acid sequences, or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, 90%, 100% of the length of the reference sequence (e.g., when aligning a second sequence to the 68730 amino acid sequence of SEQ ID NO:2 having 357 amino acid residues, at least 107, preferably at least 143, more preferably at least 179, even more preferably at least 214, and even more preferably at least 250, 286, 321, or 357 amino acid residues are aligned, or when aligning a second sequence to the 69112 amino acid sequence of SEQ ID NO:5 having 648 amino acid residues, at least 194, preferably at least 259, more preferably at least 324, even more preferably at least 389, and even more preferably at least 454, 518, 583, or 648 amino acid residues are aligned). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by

the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

[0073] The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at <http://www.gcg.com>), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at <http://www.gcg.com>), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. A particularly preferred set of parameters (and the one that should be used if the practitioner is uncertain about what parameters should be applied to determine if a molecule is within a sequence identity or homology limitation of the invention) is using a Blossum 62 scoring matrix with a gap open penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

[0074] The percent identity between two amino acid or nucleotide sequences can be determined using the algorithm of E. Meyers and W. Miller (CABIOS, 4:11-17 (1989)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

[0075] The nucleic acid and protein sequences described herein can be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, et al., (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to 68730 or 69112 nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to 68730 or 69112 protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST

and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See <http://www.ncbi.nlm.nih.gov>.

[0076] "Misexpression or aberrant expression", as used herein, refers to a non-wild type pattern of gene expression, at the RNA or protein level. It includes: expression at non-wild type levels, i.e., over or under expression; a pattern of expression that differs from wild type in terms of the time or stage at which the gene is expressed, e.g., increased or decreased expression (as compared with wild type) at a predetermined developmental period or stage; a pattern of expression that differs from wild type in terms of decreased expression (as compared with wild type) in a predetermined cell type or tissue type; a pattern of expression that differs from wild type in terms of the splicing size, amino acid sequence, post-translational modification, or biological activity of the expressed polypeptide; a pattern of expression that differs from wild type in terms of the effect of an environmental stimulus or extracellular stimulus on expression of the gene, e.g., a pattern of increased or decreased expression (as compared with wild type) in the presence of an increase or decrease in the strength of the stimulus.

[0077] "Subject", as used herein, can refer to a mammal, e.g., a human, or to an experimental or animal or disease model. The subject can also be a non-human animal, e.g., a horse, cow, goat, or other domestic animal.

[0078] A "purified preparation of cells", as used herein, refers to, in the case of plant or animal cells, an *in vitro* preparation of cells and not an entire intact plant or animal. In the case of cultured cells or microbial cells, it consists of a preparation of at least 10% and more preferably 50% of the subject cells.

[0079] Various aspects of the invention are described in further detail below.

Isolated Nucleic Acid Molecules

[0080] In one aspect, the invention provides, an isolated or purified, nucleic acid molecule that encodes a 68730 or 69112 polypeptide described herein, e.g., a full length 68730 or 69112 protein, or a fragment thereof, e.g., a biologically active portion of 68730 or 69112 protein. Also included is a nucleic acid fragment suitable for use as a hybridization probe, which can be used, e.g., to identify nucleic acid molecule encoding a polypeptide of the invention, 68730 and 69112 mRNA, and fragments suitable for use as primers, e.g., PCR primers for the amplification or mutation of nucleic acid molecules.

[0081] In one embodiment, an isolated nucleic acid molecule of the present invention includes the nucleotide sequence shown in SEQ ID NO:1, or a portion of this nucleotide sequence. In one embodiment, the nucleic acid molecule includes sequences encoding the human 68730 protein (*i.e.*, "the coding region", from nucleotides 169 to 1242 of SEQ ID NO:1, including the termination codon), as well as untranslated (*e.g.*, noncoding) sequences, *e.g.*, 5' untranslated sequence (*i.e.*, nucleotides 1 to 168 of SEQ ID NO:1) and/or 3' untranslated sequence (nucleotides 1243 to 1772 of SEQ ID NO:1). Alternatively, the nucleic acid molecule can include only the coding region of SEQ ID NO:1 (*e.g.*, nucleotides 169 to 1242 of SEQ ID NO:1, corresponding to nucleotides 1 to 1074 SEQ ID NO:3) and, *e.g.*, no flanking sequences which normally accompany the subject sequence. In another embodiment, the nucleic acid molecule encodes a sequence corresponding to the mature protein of SEQ ID NO:2.

[0082] In another embodiment, an isolated nucleic acid molecule of the present invention includes the nucleotide sequence shown in SEQ ID NO:5, or a portion of this nucleotide sequence. In one embodiment, the nucleic acid molecule includes sequences encoding the human 69112 protein (*i.e.*, "the coding region", from nucleotides 1213 to 3159 of SEQ ID NO:4, including the termination codon), as well as untranslated (*e.g.*, noncoding) sequences, *e.g.*, 5' untranslated sequence (*i.e.*, nucleotides 1 to 1212 of SEQ ID NO:4) and/or 3' untranslated sequence (*i.e.*, nucleotides 3160 to 3579 of SEQ ID NO:4). Alternatively, the nucleic acid molecule can include only the coding region of SEQ ID NO:4 (*e.g.*, nucleotides 1213 to 3159 of SEQ ID NO:4, corresponding to nucleotides 1 to 1947 SEQ ID NO:3) and, *e.g.*, no flanking sequences which normally accompany the subject sequence. In another embodiment, the nucleic acid molecule encodes a sequence corresponding to the mature protein of SEQ ID NO:5.

[0083] In another embodiment, an isolated nucleic acid molecule of the invention includes a nucleic acid molecule which is a complement of the nucleotide sequence shown in SEQ ID NO:1, 3, 4, or 6, or a portion of any of these nucleotide sequences. In other embodiments, the nucleic acid molecule of the invention is sufficiently homologous to the nucleotide sequence shown in SEQ ID NO:1, 3, 4, or 6, such that it can hybridize to the nucleotide sequence shown in SEQ ID NO:1, 3, 4, or 6, respectively, thereby forming a stable duplex.

[0084] In one embodiment, an isolated nucleic acid molecule of the present invention includes a nucleotide sequence which is at least about 60%, 65%, 70%, 75%, 80%, 85%,

90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more homologous to the nucleotide sequence shown in SEQ ID NO:1, 3, 4, or 6. In the case of an isolated nucleic acid molecule which is longer than or equivalent in length to the reference sequence, *e.g.*, SEQ ID NO:1, 3, 4, or 6, the comparison is made with the full length of the reference sequence. Where the isolated nucleic acid molecule is shorter than the reference sequence, *e.g.*, shorter than SEQ ID NO:1, 3, 4, or 6, the comparison is made to a segment of the reference sequence of the same length (excluding any loop required by the homology calculation).

68730 and 69112 Nucleic Acid Fragments

[0085] A nucleic acid molecule of the invention can include only a portion of the nucleic acid sequence of SEQ ID NO:1, 3, 4, or 6. For example, such a nucleic acid molecule can include a fragment which can be used as a probe or primer or a fragment encoding a portion of a 68730 or 69112 protein, *e.g.*, an immunogenic or biologically active portion of a 68730 or 69112 protein. A fragment can comprise: nucleotides 234 to 1005 of SEQ ID NO:1, which encodes a protein kinase domain of human 68730, *e.g.*, amino acid residues 23 to 279 of SEQ ID NO:2; or nucleotides 2278 to 3051 of SEQ ID NO:4, which encodes a protein kinase domain of human 69112, *e.g.*, amino acid residues 356 to 613 of SEQ ID NO:5. The nucleotide sequences determined from the cloning of the 68730 and 69112 genes allow for the generation of probes and primers designed for use in identifying and/or cloning other family members, or fragments thereof, as well as homologues, or fragments thereof, of 68730 and 69112 genes from other species.

[0086] In another embodiment, a nucleic acid includes a nucleotide sequence that includes part, or all, of the coding region and extends into either (or both) the 5' or 3' untranslated region. Other embodiments include a fragment which includes a nucleotide sequence encoding an amino acid fragment described herein. Nucleic acid fragments can encode a specific domain or site described herein or fragments thereof, particularly fragments thereof which are at least 150 amino acids in length. Fragments also include nucleic acid sequences corresponding to specific amino acid sequences described above or fragments thereof. Nucleic acid fragments should not be construed as encompassing those fragments that may have been disclosed prior to the invention.

[0087] A nucleic acid fragment can include a sequence corresponding to a domain, region, or functional site described herein. A nucleic acid fragment can also include one or

more domain, region, or functional site described herein. Thus, for example, the nucleic acid fragment can include a protein kinase domain. In a preferred embodiment the fragment is at least, 50, 100, 200, 300, 400, 500, 600, 700, or 900 base pairs in length.

[0088] 68730 and 69112 probes and primers are provided. Typically a probe/primer is an isolated or purified oligonucleotide. The oligonucleotide typically includes a region of nucleotide sequence that hybridizes under stringent conditions to at least about 7, 12 or 15, preferably about 20 or 25, more preferably about 30, 35, 40, 45, 50, 55, 60, 65, or 75 consecutive nucleotides of a sense or antisense sequence of SEQ ID NO:1, 3, 4, or 6, or of a naturally occurring allelic variant or mutant of SEQ ID NO:1, 3, 4, or 6.

[0089] In a preferred embodiment the nucleic acid is a probe which is at least 5 or 10, and less than 200, more preferably less than 100, or less than 50, base pairs in length. It should be identical, or differ by 1, or less than in 5 or 10 bases, from a sequence disclosed herein. If alignment is needed for this comparison the sequences should be aligned for maximum homology. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences.

[0090] A probe or primer can be derived from the sense or anti-sense strand of a nucleic acid which encodes a protein kinase domain (e.g., at about nucleotides 234 to 1005 of SEQ ID NO:1, and at about nucleotides 2278 to 3051 of SEQ ID NO:4), or a fragment thereof.

[0091] In another embodiment a set of primers is provided, e.g., primers suitable for use in a PCR, which can be used to amplify a selected region of a 68730 or 69112 sequence, e.g., a region described herein. The primers should be at least 5, 10, or 50 base pairs in length and less than 100, or less than 200, base pairs in length. The primers should be identical, or differ by one base from a sequence disclosed herein or from a naturally occurring variant. For example, primers suitable for amplifying all or a portion of any of the following regions are provided: a protein kinase ATP binding domain (e.g., at about nucleotides 253 to 339 of SEQ ID NO:1); and a serine/threonine protein kinase active site signature sequence (e.g., at about nucleotides 586 to 624 of SEQ ID NO:1 and at about nucleotides 2629 to 2667 of SEQ ID NO:4).

[0092] A nucleic acid fragment can encode an epitope bearing region of a polypeptide described herein.

[0093] A nucleic acid fragment encoding a "biologically active portion of a 68730 polypeptide" or a "biologically active portion of a 69112 polypeptide" can be prepared by

isolating a portion of the nucleotide sequence of SEQ ID NO:1, 3, 4, or 6, which encodes a polypeptide having a 68730 or 69112 biological activity (e.g., the biological activities of the 68730 or 69112 proteins as described herein), expressing the encoded portion of the 68730 or 69112 protein (e.g., by recombinant expression *in vitro*) and assessing the activity of the encoded portion of the 68730 or 69112 protein. For example, a nucleic acid fragment encoding a biologically active portion of 68730 or 69112 includes a protein kinase domain (e.g., at about nucleotides 235 to 1005 of SEQ ID NO:1 or nucleotides 2278 to 3051 of SEQ ID NO:4). A nucleic acid fragment encoding a biologically active portion of a 68730 or 69112 polypeptide, can comprise a nucleotide sequence which is greater than 200-1200 or more nucleotides in length.

[0094] In preferred embodiments, nucleic acids include a nucleotide sequence which is about 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200 nucleotides in length and hybridizes under stringent hybridization conditions to a nucleic acid molecule of SEQ ID NO:1, 3, 4, or 6, or a complement thereof.

68730 and 69112 Nucleic Acid Variants

[0095] The invention further encompasses nucleic acid molecules that differ from the nucleotide sequence shown in SEQ ID NO:1, 3, 4, or 6. Such differences can be due to degeneracy of the genetic code (and result in a nucleic acid which encodes the same 68730 or 69112 proteins as those encoded by the nucleotide sequence disclosed herein. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence which differs, by at least 1, but less than 5, 10, 20, 50, or 100 amino acid residues that shown in SEQ ID NO:2 or 5. If alignment is needed for this comparison the sequences should be aligned for maximum homology. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences.

[0096] Nucleic acids of the present invention can be chosen for having codons, which are preferred, or non preferred, for a particular expression system. For example, the nucleic acid can be one in which at least one codon, and preferably at least 10%, or 20% of the codons have been altered such that the sequence is optimized for expression in bacterial (e.g., *E. coli*), yeast, human, insect, or nonmammalian cells (e.g., Chinese hamster ovary cells).

[0097] Nucleic acid variants can be naturally occurring, such as allelic variants (same locus), homologs (different locus), and orthologs (different organism) or can be non-naturally occurring. Non-naturally occurring variants can be made by mutagenesis techniques, including those applied to polynucleotides, cells, or organisms. The variants can contain nucleotide substitutions, deletions, inversions and insertions. Variation can occur in either or both the coding and non-coding regions. The variations can produce both conservative and non-conservative amino acid substitutions (as compared to the encoded product).

[0098] In a preferred embodiment, the nucleic acid differs from that of SEQ ID NO:1, 3, 4, or 6, e.g., as follows: by at least one but less than 10, 20, 30, or 40 nucleotides; at least one but less than 1%, 5%, 10% or 20% of the subject nucleic acid. If necessary for this analysis the sequences should be aligned for maximum homology. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences.

[0099] Orthologs, homologs, and allelic variants can be identified using methods known in the art. These variants comprise a nucleotide sequence encoding a polypeptide that is 50%, at least about 55%, typically at least about 70-75%, more typically at least about 80-85%, and most typically at least about 90-95% or more identical to the amino acid sequence shown in SEQ ID NO:2 or a fragment of this sequence. Such nucleic acid molecules can readily be obtained as being able to hybridize under stringent conditions, to the nucleotide sequence shown in SEQ ID NO:3 or a fragment of this sequence. Nucleic acid molecules corresponding to orthologs, homologs, and allelic variants of the 68730 and 69112 cDNAs of the invention can further be isolated by mapping to the same chromosome or locus as the 68730 or 69112 gene. Preferred variants include those that are correlated with protein kinase activity.

[00100] Allelic variants of 68730 and 69112, e.g., human 68730 and 69112, include both functional and non-functional proteins. Functional allelic variants are naturally occurring amino acid sequence variants of the 68730 and 69112 proteins within a population that maintain the ability to modulate the phosphorylation state of itself or another protein or polypeptide. Functional allelic variants will typically contain only conservative substitution of one or more amino acids of SEQ ID NO:2 or 5, or substitution, deletion or insertion of non-critical residues in non-critical regions of the protein. Non-functional allelic variants are naturally-occurring amino acid sequence variants of the 68730 and 69112, e.g., human 68730 and human 69112, protein within a

population that do not have the ability to activate signal transduction. Non-functional allelic variants will typically contain a non-conservative substitution, a deletion, or insertion, or premature truncation of the amino acid sequence of SEQ ID NO:2 or 5, or a substitution, insertion, or deletion in critical residues or critical regions of the protein.

[00101] Moreover, nucleic acid molecules encoding other 68730 and 69112 family members and, thus, which have a nucleotide sequence which differs from the 68730 and 69112 sequences of SEQ ID NO:1, 3, 4, or 6 are intended to be within the scope of the invention.

Antisense Nucleic Acid Molecules, Ribozymes and Modified 68730 and 69112 Nucleic Acid Molecules

[00102] In another aspect, the invention features, an isolated nucleic acid molecule which is antisense to 68730 or 69112. An "antisense" nucleic acid can include a nucleotide sequence which is complementary to a "sense" nucleic acid encoding a protein, e.g., complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. The antisense nucleic acid can be complementary to an entire 68730 or 69112 coding strand, or to only a portion thereof (e.g., the coding region of human 68730 corresponding to SEQ ID NO:3 or the coding region of human 69112 corresponding to SEQ ID NO:6). In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding 68730 or 69112 (e.g., the 5' and 3' untranslated regions).

[00103] An antisense nucleic acid can be designed such that it is complementary to the entire coding region of 68730 or 69112 mRNA, but more preferably is an oligonucleotide which is antisense to only a portion of the coding or noncoding region of 68730 or 69112 mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of 68730 or 69112 mRNA, e.g., between the -10 and +10 regions of the target gene nucleotide sequence of interest. An antisense oligonucleotide can be, for example, about 7, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, or more nucleotides in length.

[00104] An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides

designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used. The antisense nucleic acid also can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

[00105] The antisense nucleic acid molecules of the invention are typically administered to a subject (e.g., by direct injection at a tissue site), or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a 68730 or 69112 protein to thereby inhibit expression of the protein, e.g., by inhibiting transcription and/or translation. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, e.g., by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

[00106] In yet another embodiment, the antisense nucleic acid molecule of the invention is an α -anomeric nucleic acid molecule. An α -anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β -units, the strands run parallel to each other (Gaultier et al., (1987) *Nucleic Acids Res.* 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-O-methylribonucleotide (Inoue et al., (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue et al., (1987) *FEBS Lett.* 215:327-330).

[00107] In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. A ribozyme having specificity for a 68730- or 69112-encoding nucleic acid can include one or more sequences complementary to the nucleotide sequence of a 68730 or 69112 cDNA disclosed herein (i.e., SEQ ID NO:1, 3, 4, or 6), and a sequence having known catalytic sequence responsible for mRNA cleavage (see U.S. Pat. No. 5,093,246 or Haselhoff and Gerlach, (1988) *Nature* 334:585-591). For example, a derivative of a

Tetrahymena L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a 68730- or 69112-encoding mRNA. See, e.g., Cech et al. U.S. Patent No. 4,987,071; and Cech et al. U.S. Patent No. 5,116,742. Alternatively, 68730 or 69112 mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, e.g., Bartel, D. and Szostak, J.W. (1993) *Science* 261:1411-1418.

[00108] 68730 and 69112 gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the 68730 or 69112 (e.g., the 68730 or 69112 promoter and/or enhancers) to form triple helical structures that prevent transcription of the 68730 or 69112 gene in target cells. See generally, Helene, C., (1991) *Anticancer Drug Des.* 6(6):569-84; Helene, C. et al., (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher, L.J., (1992) *Bioassays* 14(12):807-15. The potential sequences that can be targeted for triple helix formation can be increased by creating a so-called "switchback" nucleic acid molecule. Switchback molecules are synthesized in an alternating 5'-3', 3'-5' manner, such that they base pair with first one strand of a duplex and then the other, eliminating the necessity for a sizeable stretch of either purines or pyrimidines to be present on one strand of a duplex.

[00109] The invention also provides detectably labeled oligonucleotide primer and probe molecules. Typically, such labels are chemiluminescent, fluorescent, radioactive, or colorimetric.

[00110] A 68730 and a 69112 nucleic acid molecule can be modified at the base moiety, sugar moiety or phosphate backbone to improve, e.g., the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acid molecules can be modified to generate peptide nucleic acids (see Hyrup B. et al., (1996) *Bioorganic & Medicinal Chemistry* 4 (1): 5-23). As used herein, the terms "peptide nucleic acid" or "PNA" refers to a nucleic acid mimic, e.g., a DNA mimic, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of a PNA can allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup B. et al., (1996) *supra*; Perry-O'Keefe et al., *Proc. Natl. Acad. Sci.* 93: 14670-675.

[00111] PNAs of 68730 and 69112 nucleic acid molecules can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, for example, inducing transcription or translation arrest or inhibiting replication. PNAs of 68730 and 69112 nucleic acid molecules can also be used in the analysis of single base pair mutations in a gene, (e.g., by PNA-directed PCR clamping); as 'artificial restriction enzymes' when used in combination with other enzymes, (e.g., S1 nucleases (Hyrup B., (1996) *supra*)); or as probes or primers for DNA sequencing or hybridization (Hyrup B. et al., (1996) *supra*; Perry-O'Keefe *supra*).

[00112] In other embodiments, the oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al., (1989) *Proc. Natl. Acad. Sci. USA* 86:6553-6556; Lemaitre et al., (1987) *Proc. Natl. Acad. Sci. USA* 84:648-652; PCT Publication No. W088/09810) or the blood-brain barrier (see, e.g., PCT Publication No. W089/10134). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (See, e.g., Krol et al., (1988) *Bio-Techniques* 6:958-976) or intercalating agents. (See, e.g., Zon, (1988) *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, (e.g., a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent).

[00113] The invention also includes molecular beacon oligonucleotide primer and probe molecules having at least one region which is complementary to a 68730 or 69112 nucleic acid of the invention, two complementary regions one having a fluorophore and one a quencher such that the molecular beacon is useful for quantitating the presence of the 68730 or 69112 nucleic acid of the invention in a sample. Molecular beacon nucleic acids are described, for example, in Lizardi et al., U.S. Patent No. 5,854,033; Nazarenko et al., U.S. Patent No. 5,866,336, and Livak et al., U.S. Patent 5,876,930.

Isolated 68730 and 69112 Polypeptides

[00114] In another aspect, the invention features, isolated 68730 and 69112 proteins, or fragments, e.g., biologically active portions, for use as immunogens or antigens to raise or test (or more generally to bind) anti-68730 and anti-69112 antibodies. 68730 and 69112 proteins can be isolated from cells or tissue sources using standard protein purification

techniques. 68730 and 69112 proteins, or fragments thereof, can be produced by recombinant DNA techniques or synthesized chemically.

[00115] Polypeptides of the invention include those which arise as a result of the existence of multiple genes, alternative transcription events, alternative RNA splicing events, and alternative translational and postranslational events. The polypeptide can be expressed in systems, e.g., cultured cells, which result in a polypeptide with substantially the same postranslational modifications as the polypeptide expressed in a native host cell, or in systems which result in the alteration or omission of postranslational modifications, e.g., glycosylation or cleavage, which otherwise occur when the polypeptide is expressed in its native host cell.

[00116] In a preferred embodiment, a 68730 or 69112 polypeptide has one or more of the following characteristics:

- the ability to act as a protein kinase or to activate a protein kinase activity;
- the ability to act as a substrate for a protein kinase, *e.g.*, a tyrosine kinase;
- a molecular weight, *e.g.*, a deduced molecular weight, amino acid composition or other physical characteristic of the polypeptide of SEQ ID NO:2 or 5;
- an overall sequence similarity of at least 50%, preferably at least 60%, more preferably at least 70, 80, 90, or 95%, with a polypeptide of SEQ ID NO:2 or 5;
- a protein kinase domain which preferably has an overall sequence similarity of about 70%, 80%, 90% or 95% with amino acid residues 23 to 279 of SEQ ID NO:2 or amino acid residues 356 to 613 of SEQ ID NO:5; and
- the presence of at least 70%, preferably 80%, and most preferably 95% of the cysteines found in the amino acid sequence of the 68730 or 69112 native protein.

[00117] In a preferred embodiment, the 68730 or 69112 protein, or a fragment thereof, differs from the corresponding sequence in SEQ ID NO:2 or 5. In one embodiment it differs by at least one but by less than 15, 10 or 5 amino acid residues. In another it differs from the corresponding sequence in SEQ ID NO:2 or 5 by at least one residue but less than 20%, 15%, 10% or 5% of the residues in it differ from the corresponding sequence in SEQ ID NO:2 or 5. (If this comparison requires alignment the sequences should be aligned for maximum homology. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences.) The differences are, preferably, differences or changes at a non-essential residue or a conservative substitution. In a preferred embodiment the differences are not in the protein kinase domain. In another

preferred embodiment one or more differences are in non-active site residues, e.g. outside of the protein kinase domain.

[00118] Other embodiments include a protein that contain one or more changes in amino acid sequence, e.g., a change in an amino acid residue which is not essential for activity. Such 68730 and 69112 proteins differ in amino acid sequence from SEQ ID NO:2 and 5, respectively, yet retain biological activity.

[00119] In one embodiment, a biologically active portion of a 68730 and 69112 protein includes a protein kinase domain. Moreover, other biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the biological activities of a native 68730 and 69112 protein.

[00120] In a preferred embodiment, the 68730 protein has an amino acid sequence shown in SEQ ID NO:2. In other embodiments, the 68730 protein is sufficiently identical to SEQ ID NO:2. In yet another embodiment, the 68730 protein is sufficiently identical to SEQ ID NO:2 and retains the biological activity of the protein of SEQ ID NO:2, as described in detail above. Accordingly, in another embodiment, the 68730 protein is a protein which includes an amino acid sequence at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or more identical to SEQ ID NO:2.

[00121] In another preferred embodiment, the 69112 protein has an amino acid sequence shown in SEQ ID NO:5. In other embodiments, the 69112 protein is sufficiently identical to SEQ ID NO:5. In yet another embodiment, the 69112 protein is sufficiently identical to SEQ ID NO:5 and retains the biological activity of the protein of SEQ ID NO:5, as described in detail above. Accordingly, in another embodiment, the 68730 protein is a protein which includes an amino acid sequence at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or more identical to SEQ ID NO:5.

68730 and 69112 Chimeric or Fusion Proteins

[00122] In another aspect, the invention provides 68730 and 69112 chimeric or fusion proteins. As used herein, a 68730 and 69112 "chimeric protein" or "fusion protein" includes a 68730 or 69112 polypeptide linked to a non-68730 or non-69112 polypeptide. A "non-68730 polypeptide" and "non-69112 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein which is not sufficiently homologous to the 68730 or 69112 protein, e.g., a protein which is different from the 68730 or 69112 protein and which is derived from the same or a different organism. The 68730 or

polypeptide of the fusion protein can correspond to all or a portion e.g., a fragment described herein of a 68730 or 69112 amino acid sequence. In a preferred embodiment, a 68730 or 69112 fusion protein includes at least one (or two) biologically active portion of a 68730 or 69112 protein. The non-68730 or non-69112 polypeptide can be fused to the N-terminus or C-terminus of the 68730 or 69112 polypeptide.

[00123] The fusion protein can include a moiety which has a high affinity for a ligand. For example, the fusion protein can be a GST-68730 or GST-69112 fusion protein in which the 68730 or 69112 sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of recombinant 68730 or 69112. Alternatively, the fusion protein can be a 68730 or 69112 protein containing a heterologous signal sequence at its N-terminus. In certain host cells (e.g., mammalian host cells), expression and/or secretion of 68730 or 69112 can be increased through use of a heterologous signal sequence.

[00124] Fusion proteins can include all or a part of a serum protein, e.g., a portion of an immunoglobulin (e.g., IgG, IgA, or IgE), e.g., an Fc region and/or the hinge C1 and C2 sequences of an immunoglobulin or human serum albumin.

[00125] The 68730 and 69112 fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject *in vivo*. The 68730 and 69112 fusion proteins can be used to affect the bioavailability of a 68730 or 69112 substrate. 68730 and 69112 fusion proteins can be useful therapeutically for the treatment of disorders caused by, for example, (i) aberrant modification or mutation of a gene encoding a 68730 or 69112 protein; (ii) mis-regulation of the 68730 or 69112 gene; and (iii) aberrant post-translational modification of a 68730 or 69112 protein.

[00126] Moreover, the 68730 and 69112 fusion proteins of the invention can be used as immunogens to produce anti-68730 and anti-69112 antibodies in a subject, to purify 68730 and 69112 ligands, and in screening assays, to identify molecules which inhibit the interaction of 68730 with a 68730 substrate and 69112 with a 69112 substrate.

[00127] Expression vectors are commercially available that already encode a fusion moiety (e.g., a GST polypeptide). A 68730- or 69112-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the 68730 or 69112 protein.

Variants of 68730 and 69112 Proteins

[00128] In another aspect, the invention also features a variant of a 68730 and a 69112 polypeptide, e.g., which functions as an agonist (mimetics) or as an antagonist. Variants of the 68730 and 69112 proteins can be generated by mutagenesis, e.g., discrete point mutation, the insertion or deletion of sequences or the truncation of a 68730 or a 69112 protein. An agonist of the 68730 and 69112 proteins can retain sufficiently the same, or a subset, of the biological activities of the naturally occurring form of a 68730 or 69112 protein. An antagonist of a 68730 or 69112 protein can inhibit one or more of the activities of the naturally occurring form of the 68730 or 69112 protein by, for example, competitively modulating a 68730- or 69112-mediated activity of a 68730 or 69112 protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. Preferably, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the 68730 or 69112 protein.

[00129] Variants of a 68730 or 69112 protein can be identified by screening combinatorial libraries of mutants, e.g., truncation mutants, of a 68730 or 69112 protein for agonist or antagonist activity.

[00130] Libraries of fragments e.g., N terminal, C terminal, or internal fragments, of a 68730 or 69112 protein coding sequence can be used to generate a variegated population of fragments for screening and subsequent selection of variants of a 68730 or 69112 protein.

[00131] Variants in which a cysteine residues is added or deleted or in which a residue which is glycosylated is added or deleted are particularly preferred.

[00132] Methods for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property are known in the art. Such methods are adaptable for rapid screening of the gene libraries generated by combinatorial mutagenesis of 68730 and 69112 proteins. Recursive ensemble mutagenesis (REM), a new technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify 68730 and 69112 variants (Arkin and Yourvan, (1992) *Proc. Natl. Acad. Sci. USA* 89:7811-7815; Delgrave et al., (1993) *Protein Engineering* 6(3):327-331).

[00133] Cell based assays can be exploited to analyze a variegated 68730 or 69112 library. For example, a library of expression vectors can be transfected into a cell line, e.g., a cell line, which ordinarily responds to 68730 or 69112 in a substrate-dependent manner. The transfected cells are then contacted with 68730 or 69112 and the effect of the expression of the mutant on signaling by the respective 68730 or 69112 substrate can be detected, e.g., by measuring protein kinase activity. Plasmid DNA can then be recovered from the cells which score for inhibition, or alternatively, potentiation of signaling by the 68730 or 69112 substrate, and the individual clones further characterized.

[00134] In another aspect, the invention features a method of making a 68730 and a 69112 polypeptide, e.g., a peptide having a non-wild type activity, e.g., an antagonist, agonist, or super agonist of a naturally occurring 68730 or 69112 polypeptide, e.g., a naturally occurring 68730 or 69112 polypeptide. The method includes: altering the sequence of a 68730 or 69112 polypeptide, e.g., altering the sequence, e.g., by substitution or deletion of one or more residues of a non-conserved region, a domain or residue disclosed herein, and testing the altered polypeptide for the desired activity.

[00135] In another aspect, the invention features a method of making a fragment or analog of a 68730 and a 69112 polypeptide having a biological activity of a naturally occurring 68730 or 69112 polypeptide. The method includes: altering the sequence, e.g., by substitution or deletion of one or more residues, of a 68730 or 69112 polypeptide, e.g., altering the sequence of a non-conserved region, or a domain or residue described herein, and testing the altered polypeptide for the desired activity.

Anti-68730 and Anti-69112 Antibodies

[00136] In another aspect, the invention provides an anti-68730 and an anti-69112 antibody. The term "antibody" as used herein refers to an immunoglobulin molecule or immunologically active portion thereof, i.e., an antigen-binding portion. Examples of immunologically active portions of immunoglobulin molecules include scFV and dcFV fragments, Fab and F(ab')₂ fragments which can be generated by treating the antibody with an enzyme such as pepsin.

[00137] The antibody can be a polyclonal, monoclonal, recombinant, e.g., a chimeric, humanized, fully human, non-human (e.g., murine, rat, rabbit, or goat), or single chain

antibody. In a preferred embodiment it has effector function and can fix complement. The antibody can be coupled to a toxin or imaging agent.

[00138] A full-length 68730 or 69112 protein, or an antigenic peptide fragment of 68730 or 69112, can be used as an immunogen or can be used to identify anti-68730 or anti-69112 antibodies made with other immunogens, e.g., cells, membrane preparations, and the like. The antigenic peptides of 68730 and 69112 should include at least 8 amino acid residues of the amino acid sequence shown in SEQ ID NO:2 or 5 and encompass an epitope of 68730 or 69112, respectively. Preferably, the antigenic peptide includes at least 10 amino acid residues, more preferably at least 15 amino acid residues, even more preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues.

[00139] Fragments of 68730 and 69112 which include, e.g., residues 23 to 279 of SEQ ID NO:2 and residues 356 to 613 of SEQ ID NO:5, respectively, can be used as immunogens to make an antibody against what is believed to be the protein kinase region of the 68730 and 69112 protein.

[00140] Antibodies reactive with, or specific for, any of these regions, or other regions or domains described herein are provided.

[00141] In an alternative embodiment the antibody fails to bind to an Fc receptor, e.g., it is a type which does not support Fc receptor binding or has been modified, e.g., by deletion or other mutation, such that it does not have a functional Fc receptor binding region.

[00142] Preferred epitopes encompassed by the antigenic peptide are regions of 68730 and 69112 which are located on the surface of the protein, e.g., hydrophilic regions (depicted, e.g., in the hydropathy plots in Figures 1 and 2, as residues below the dashed horizontal line), as well as regions with high antigenicity. For example, an Emini surface probability analysis of the human 68730 and 69112 protein sequences can be used to indicate the regions that have a particularly high probability of being localized to the surface of the 68730 and 69112 protein, respectively, and are thus likely to constitute surface residues useful for targeting antibody production.

[00143] In a preferred embodiment the antibody binds an epitope on any domain or region on 68730 and 69112 proteins described herein.

[00144] Chimeric, humanized, but most preferably, completely human antibodies are desirable for applications which include repeated administration, e.g., therapeutic treatment (and some diagnostic applications) of human patients.

[00145] The anti-68730 and anti-69112 antibodies can each be a single chain antibody. A single-chain antibody (scFV) may be engineered as described, for example, in Colcher, D. et al., *Ann. NY Acad. Sci.* 1999 Jun 30;880:263-80; and Reiter, Y., *Clin. Cancer Res.* 1996 Feb;2(2):245-52. The single chain antibody can be dimerized or multimerized to generate multivalent antibodies having specificities for different epitopes of the same target 68730 and 69112 protein.

[00146] Anti-68730 and anti-69112 antibodies (e.g., monoclonal antibodies) can be used to isolate 68730 and 69112, respectively, by standard techniques, such as affinity chromatography or immunoprecipitation. Moreover, an anti-68730 or anti-69112 antibody can be used to detect 68730 or 69112 protein, respectively, (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the protein. Anti-68730 and anti-69112 antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, e.g., to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (i.e., physically linking) the antibody to a detectable substance (i.e., antibody labeling). Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{35}S or ^3H .

Recombinant Expression Vectors, Host Cells and Genetically Engineered Cells

[00147] In another aspect, the invention includes, vectors, preferably expression vectors, containing a nucleic acid encoding a polypeptide described herein. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another

nucleic acid to which it has been linked and can include a plasmid, cosmid or viral vector. The vector can be capable of autonomous replication or it can integrate into a host DNA. Viral vectors include, e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses.

[00148] A vector can include a 68730 or 69112 nucleic acid in a form suitable for expression of the nucleic acid in a host cell. Preferably the recombinant expression vector includes one or more regulatory sequences operatively linked to the nucleic acid sequence to be expressed. The term "regulatory sequence" includes promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Regulatory sequences include those which direct constitutive expression of a nucleotide sequence, as well as tissue-specific regulatory and/or inducible sequences. The design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, and the like. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or polypeptides, including fusion proteins or polypeptides, encoded by nucleic acids as described herein (e.g., 68730 and 69112 proteins, mutant forms of 68730 and 69112 proteins, fusion proteins, and the like).

[00149] The recombinant expression vectors of the invention can be designed for expression of 68730 or 69112 proteins in prokaryotic or eukaryotic cells. For example, polypeptides of the invention can be expressed in bacterial cells (e.g., *E. coli*), insect cells (e.g., using baculovirus expression vectors), yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

[00150] Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant

protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B. and Johnson, K.S., (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

[00151] Purified fusion proteins can be used in 68730 and 69112 activity assays, (e.g., direct assays or competitive assays described in detail below), or to generate antibodies specific for 68730 and 69112 proteins. In a preferred embodiment, a fusion protein expressed in a retroviral expression vector of the present invention can be used to infect bone marrow cells which are subsequently transplanted into irradiated recipients. The pathology of the subject recipient is then examined after sufficient time has passed (e.g., six (6) weeks).

[00152] To maximize recombinant protein expression in *E. coli* is to express the protein in host bacteria with an impaired capacity to proteolytically cleave the recombinant protein (Gottesman, S., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 119-128). Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wada et al., (1992) *Nucleic Acids Res.* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

[00153] The 68730 and 69112 expression vectors can be a yeast expression vector, a vector for expression in insect cells, e.g., a baculovirus expression vector, or a vector suitable for expression in mammalian cells.

[00154] When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40.

[00155] In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid). Non-limiting examples of suitable tissue-specific promoters include the albumin promoter

(liver-specific; Pinkert et al., (1987) *Genes Dev.* 1:268-277), lymphoid-specific promoters (Calame and Eaton, (1988) *Adv. Immunol.* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore, (1989) *EMBO J.* 8:729-733) and immunoglobulins (Banerji et al., (1983) *Cell* 33:729-740; Queen and Baltimore, (1983) *Cell* 33:741-748), neuron-specific promoters (e.g., the neurofilament promoter; Byrne and Ruddle, (1989) *Proc. Natl. Acad. Sci. USA* 86:5473-5477), pancreas-specific promoters (Edlund et al., (1985) *Science* 230:912-916), and mammary gland-specific promoters (e.g., milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example, the murine hox promoters (Kessel and Gruss, (1990) *Science* 249:374-379) and the α -fetoprotein promoter (Campes and Tilghman, (1989) *Genes Dev.* 3:537-546).

[00156] The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. Regulatory sequences (e.g., viral promoters and/or enhancers) operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the constitutive, tissue specific or cell type specific expression of antisense RNA in a variety of cell types. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus. For a discussion of the regulation of gene expression using antisense genes see Weintraub, H. et al., Antisense RNA as a molecular tool for genetic analysis, *Reviews - Trends in Genetics*, Vol. 1(1) 1986.

[00157] Another aspect the invention provides a host cell which includes a nucleic acid molecule described herein, e.g., a 68730 or 69112 nucleic acid molecule within a recombinant expression vector or a 68730 or 69112 nucleic acid molecule containing sequences which allow it to homologously recombine into a specific site of the host cell's genome. The terms "host cell" and "recombinant host cell" are used interchangeably herein. Such terms refer not only to the particular subject cell but rather also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

[00158] A host cell can be any prokaryotic or eukaryotic cell. For example, a 68730 or 69112 protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or

mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

[00159] Vector DNA can be introduced into host cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (e.g., DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation.

[00160] A host cell of the invention can be used to produce (i.e., express) a 68730 or a 69112 protein. Accordingly, the invention further provides methods for producing 68730 and 69112 proteins using the host cells of the invention. In one embodiment, the method includes culturing the host cell of the invention (into which a recombinant expression vector encoding a 68730 or 69112 protein has been introduced) in a suitable medium such that a 68730 or a 69112 protein is produced. In another embodiment, the method further includes isolating a 68730 or a 69112 protein from the medium or the host cell.

[00161] In another aspect, the invention features, a cell or purified preparation of cells which includes a 68730 and a 69112 transgene, or which otherwise misexpresses 68730 or 69112. The cell preparation can consist of human or non-human cells, e.g., rodent cells, e.g., mouse or rat cells, rabbit cells, or pig cells. In preferred embodiments, the cell or cells include a 68730 and a 69112 transgene, e.g., a heterologous form of a 68730 and a 69112, e.g., a gene derived from humans (in the case of a non-human cell). The 68730 or 69112 transgene can be misexpressed, e.g., overexpressed or underexpressed. In other preferred embodiments, the cell or cells include a gene which misexpress an endogenous 68730 or 69112, e.g., a gene the expression of which is disrupted, e.g., a knockout. Such cells can serve as a model for studying disorders which are related to mutated or mis-expressed 68730 or 69112 alleles or for use in drug screening.

[00162] In another aspect, the invention features, a human cell, e.g., a hematopoietic stem cell, transformed with nucleic acid which encodes a subject 68730 or 69112 polypeptide.

[00163] Also provided are cells or a purified preparation thereof, e.g., human cells, in which an endogenous 68730 or 69112 is under the control of a regulatory sequence that does not normally control the expression of the endogenous 68730 or 69112 gene. The expression characteristics of an endogenous gene within a cell, e.g., a cell line or microorganism, can be modified by inserting a heterologous DNA regulatory element into

the genome of the cell such that the inserted regulatory element is operably linked to the endogenous 68730 or 69112 gene. For example, an endogenous 68730 or 69112 gene, e.g., a gene which is "transcriptionally silent," e.g., not normally expressed, or expressed only at very low levels, may be activated by inserting a regulatory element which is capable of promoting the expression of a normally expressed gene product in that cell. Techniques such as targeted homologous recombinations, can be used to insert the heterologous DNA as described in, e.g., Chappel, US 5,272,071; WO 91/06667, published on May 16, 1991.

Transgenic Animals

[00164] The invention provides non-human transgenic animals. Such animals are useful for studying the function and/or activity of a 68730 or 69112 protein and for identifying and/or evaluating modulators of 68730 or 69112 activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, and the like. A transgene is exogenous DNA or a rearrangement, e.g., a deletion of endogenous chromosomal DNA, which preferably is integrated into or occurs in the genome of the cells of a transgenic animal. A transgene can direct the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal, other transgenes, e.g., a knockout, reduce expression. Thus, a transgenic animal can be one in which an endogenous 68730 or 69112 gene has been altered by, e.g., by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, e.g., an embryonic cell of the animal, prior to development of the animal.

[00165] Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to a transgene of the invention to direct expression of a 68730 or 69112 protein to particular cells. A transgenic founder animal can be identified based upon the presence of a 68730 or 69112 transgene in its genome and/or expression of 68730 or 69112 mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the

transgene. Moreover, transgenic animals carrying a transgene encoding a 68730 or 69112 protein can further be bred to other transgenic animals carrying other transgenes.

[00166] 68730 and 69112 proteins or polypeptides can be expressed in transgenic animals or plants, e.g., a nucleic acid encoding the protein or polypeptide can be introduced into the genome of an animal. In preferred embodiments the nucleic acid is placed under the control of a tissue specific promoter, e.g., a milk or egg specific promoter, and recovered from the milk or eggs produced by the animal. Suitable animals are mice, pigs, cows, goats, and sheep.

[00167] The invention also includes a population of cells from a transgenic animal, as discussed herein.

Uses

[00168] The nucleic acid molecules, proteins, protein homologues, and antibodies described herein can be used in one or more of the following methods: a) screening assays; b) predictive medicine (e.g., diagnostic assays, prognostic assays, monitoring clinical trials, and pharmacogenetics); and c) methods of treatment (e.g., therapeutic and prophylactic). The isolated nucleic acid molecules of the invention can be used, for example, to express a 68730 or 69112 protein (e.g., via a recombinant expression vector in a host cell in gene therapy applications), to detect a 68730 or 69112 mRNA (e.g., in a biological sample) or a genetic alteration in a 68730 or a 69112 gene, and to modulate 68730 or 69112 activity, as described further below. The 68730 and 69112 proteins can be used to treat disorders characterized by insufficient or excessive production of a 68730 or 69112 substrate or production of 68730 or 69112 inhibitors. In addition, the 68730 and 69112 proteins can be used to screen for naturally occurring 68730 and 69112 substrates, to screen for drugs or compounds which modulate 68730 or 69112 activity, as well as to treat disorders characterized by insufficient or excessive production of 68730 or 69112 protein or production of 68730 or 69112 protein forms which have decreased, aberrant or unwanted activity compared to 68730 or 69112 wild-type protein. Such disorders include those characterized by aberrant signaling or aberrant, e.g., hyperproliferative, cell growth. Moreover, the anti-68730 and anti-69112 antibodies of the invention can be used to detect and isolate 68730 or 69112 proteins, regulate the bioavailability of 68730 or 69112 proteins, and modulate 68730 or 69112 activity.

[00169] A method of evaluating a compound for the ability to interact with, e.g., bind, subject 68730 and 69112 polypeptides are provided. The method includes: contacting the compound with the subject 68730 or 69112 polypeptide; and evaluating ability of the compound to interact with, e.g., to bind or form a complex with the subject 68730 or 69112 polypeptide. This method can be performed *in vitro*, e.g., in a cell free system, or *in vivo*, e.g., in a two-hybrid interaction trap assay. This method can be used to identify naturally occurring molecules which interact with subject 68730 or 69112 polypeptides. It can also be used to find natural or synthetic inhibitors of subject 68730 or 69112 polypeptides. Screening methods are discussed in more detail below.

Screening Assays

[00170] The invention provides methods (also referred to herein as "screening assays") for identifying modulators, i.e., candidate or test compounds or agents (e.g., proteins, peptides, peptidomimetics, peptoids, small molecules or other drugs) which bind to 68730 or 69112 proteins, have a stimulatory or inhibitory effect on, for example, 68730 or 69112 expression, or 68730 or 69112 activity, or have a stimulatory or inhibitory effect on, for example, the expression or activity of a 68730 or 69112 substrate. Compounds thus identified can be used to modulate the activity of target gene products (e.g., 68730 or 69112 genes) in a therapeutic protocol, to elaborate the biological function of the target gene product, or to identify compounds that disrupt normal target gene interactions.

[00171] In one embodiment, the invention provides assays for screening candidate or test compounds which are substrates of a 68730 or 69112 protein or polypeptide, or a biologically active portion thereof. In another embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of a 68730 or 69112 protein or polypeptide or a biologically active portion thereof.

[00172] The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; peptoid libraries [libraries of molecules having the functionalities of peptides, but with a novel, non-peptide backbone which are resistant to enzymatic degradation but which nevertheless remain bioactive] (see, e.g., Zuckermann, R.N. et al., *J. Med. Chem.* 1994, 37: 2678-85); spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography

selection. The biological library and peptoid library approaches are limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K.S. (1997) *Anticancer Drug Des.* 12:145).

[00173] Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6909; Erb *et al.*, (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann *et al.*, (1994). *J. Med. Chem.* 37:2678; Cho *et al.*, (1993) *Science* 261:1303; Carrell *et al.*, (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell *et al.*, (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and in Gallop *et al.*, (1994) *J. Med. Chem.* 37:1233.

[00174] Libraries of compounds may be presented in solution (e.g., Houghten, (1992) *Biotechniques* 13:412-421), or on beads (Lam, (1991) *Nature* 354:82-84), chips (Fodor, (1993) *Nature* 364:555-556), bacteria or spores (Ladner, United States Patent No. 5,223,409), plasmids (Cull *et al.*, (1992) *Proc. Natl. Acad. Sci. USA* 89:1865-1869) or on phage (Scott and Smith, (1990) *Science* 249:386-390); (Devlin, (1990) *Science* 249:404-406); (Cwirla *et al.*, (1990) *Proc. Natl. Acad. Sci.* 87:6378-6382); (Felici, (1991) *J. Mol. Biol.* 222:301-310); (Ladner, *supra*).

[00175] In another embodiment, an assay is a cell-based comprising contacting a cell expressing a 68730 or 69112 target molecule (e.g., a 68730 or 69112 phosphorylation substrate) with a test compound and determining the ability of the test compound to modulate (e.g., stimulate or inhibit) the activity of the 68730 or 69112 target molecule. Determining the ability of the test compound to modulate the activity of a 68730 or 69112 target molecule can be accomplished, for example, by determining the ability of the 68730 or 69112 protein to bind to or interact with the 68730 or 69112 target molecule, or by determining the ability of the 68730 or 69112 protein to phosphorylate the 68730 or 69112 target molecule.

[00176] The ability of the 68730 or 69112 protein to phosphorylate a substrate/target molecule can be determined by, for example, an *in vitro* kinase assay. Briefly, a substrate/target molecule, e.g., an immunoprecipitated substrate/target molecule from a cell line expressing such a molecule, can be incubated with the 68730 or 69112 protein and radioactive ATP, e.g., [γ -³²P] ATP, in a buffer containing MgCl₂ and MnCl₂, e.g., 10 mM MgCl₂ and 5 mM MnCl₂. Following the incubation, the immunoprecipitated substrate/target molecule can be separated by SDS-polyacrylamide gel electrophoresis

under reducing conditions, transferred to a membrane, *e.g.*, a PVDF membrane, and autoradiographed. The appearance of detectable bands on the autoradiograph indicates that the 68730 or 69112 substrate/target molecule has been phosphorylated. Phosphoaminoacid analysis of the phosphorylated substrate/target molecule can also be performed in order to determine which residues on the 68730 or 69112 substrate/target molecule are phosphorylated. Briefly, the radiophosphorylated protein band can be excised from the SDS gel and subjected to partial acid hydrolysis. The products can then be separated by one-dimensional electrophoresis and analyzed on, for example, a phosphoimager and compared to ninhydrin-stained phosphoaminoacid standards.

[00177] The ability of the test compound to modulate 68730 or 69112 binding to a compound, *e.g.*, a 68730 or 69112 substrate, or to bind to 68730 or 69112 can also be evaluated. This can be accomplished, for example, by coupling the compound, *e.g.*, the substrate, with a radioisotope or enzymatic label such that binding of the compound, *e.g.*, the substrate, to 68730 or 69112 can be determined by detecting the labeled compound, *e.g.*, substrate, in a complex. Alternatively, 68730 or 69112 can be coupled with a radioisotope or enzymatic label to monitor the ability of a test compound to modulate 68730 or 69112 binding to a 68730 or 69112 substrate in a complex. For example, compounds (*e.g.*, substrates) can be labeled with ^{125}I , ^{35}S , ^{14}C , or ^3H , either directly or indirectly, and the radioisotope detected by direct counting of radioemmission or by scintillation counting. Alternatively, compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

[00178] The ability of a compound (*e.g.*, a 68730 or 69112 substrate) to interact with 68730 or 69112 with or without the labeling of any of the interactants can be evaluated. For example, a microphysiometer can be used to detect the interaction of a compound with 68730 or 69112 without the labeling of either the compound or 68730 or 69112.

McConnell, H. M. et al., (1992) *Science* 257:1906-1912. As used herein, a "microphysiometer" (*e.g.*, Cytosensor) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a compound and 68730 or 69112.

[00179] In a preferred embodiment, determining the ability of the 68730 or 69112 protein to bind to or interact with a 68730 or 69112 target molecule can be accomplished

by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (e.g., intracellular Ca^{2+} , diacylglycerol, IP_3 , etc.), detecting catalytic/enzymatic activity of the target on an appropriate substrate, detecting the induction of a reporter gene (comprising a target-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, e.g., chloramphenicol acetyl transferase), or detecting a target-regulated cellular response.

[00180] In yet another embodiment, a cell-free assay is provided in which a 68730 or 69112 protein, or biologically active portion thereof, is contacted with a test compound and the ability of the test compound to bind to the 68730 or 69112 protein, or biologically active portion thereof is evaluated. Preferred biologically active portions of the 68730 and 69112 proteins to be used in assays of the present invention include fragments which participate in interactions with non-68730 or non-69112 molecules, e.g., fragments with high surface probability scores.

[00181] Soluble and/or membrane-bound forms of isolated proteins (e.g., 68730 and 69112 proteins, or biologically active portions thereof) can be used in the cell-free assays of the invention. When membrane-bound forms of the protein are used, it may be desirable to utilize a solubilizing agent. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton[®] X-100, Triton[®] X-114, Thesit[®], Isotridecypoly(ethylene glycol ether)_n, 3-[(3-cholamidopropyl)dimethylamminio]-1-propane sulfonate (CHAPS), 3-[(3-cholamidopropyl)dimethylamminio]-2-hydroxy-1-propane sulfonate (CHAPSO), or N-dodecyl-N,N-dimethyl-3-ammonio-1-propane sulfonate.

[00182] Cell-free assays involve preparing a reaction mixture of the target gene protein and the test compound under conditions and for a time sufficient to allow the two components to interact and bind, thus forming a complex that can be removed and/or detected.

[00183] In one embodiment, assays are performed where the ability of an agent to block protein kinase activity within a cell is evaluated.

[00184] The interaction between two molecules can also be detected, e.g., using fluorescence energy transfer (FET) (see, for example, Lakowicz *et al.*, U.S. Patent No. 5,631,169; Stavrianopoulos, et al., U.S. Patent No. 4,868,103). A fluorophore label on

the first, 'donor' molecule is selected such that its emitted fluorescent energy will be absorbed by a fluorescent label on a second, 'acceptor' molecule, which in turn is able to fluoresce due to the absorbed energy. Alternately, the 'donor' protein molecule may simply utilize the natural fluorescent energy of tryptophan residues. Labels are chosen that emit different wavelengths of light, such that the 'acceptor' molecule label may be differentiated from that of the 'donor'. Since the efficiency of energy transfer between the labels is related to the distance separating the molecules, the spatial relationship between the molecules can be assessed. In a situation in which binding occurs between the molecules, the fluorescent emission of the 'acceptor' molecule label in the assay should be maximal. An FET binding event can be conveniently measured through standard fluorometric detection means well known in the art (e.g., using a fluorimeter).

[00185] In another embodiment, determining the ability of the 68730 or 69112 protein to bind to a target molecule can be accomplished using real-time Biomolecular Interaction Analysis (BIA) (see, e.g., Sjolander, S. and Urbaniczky, C., (1991) *Anal. Chem.* **63**:2338-2345 and Szabo et al., (1995) *Curr. Opin. Struct. Biol.* **5**:699-705). "Surface plasmon resonance" or "BIA" detects biospecific interactions in real time, without labeling any of the interactants (e.g., BIAcore). Changes in the mass at the binding surface (indicative of a binding event) result in alterations of the refractive index of light near the surface (the optical phenomenon of surface plasmon resonance (SPR)), resulting in a detectable signal which can be used as an indication of real-time reactions between biological molecules.

[00186] In one embodiment, the target gene product or the test substance is anchored onto a solid phase. The target gene product/test compound complexes anchored on the solid phase can be detected at the end of the reaction. Preferably, the target gene product can be anchored onto a solid surface, and the test compound, (which is not anchored), can be labeled, either directly or indirectly, with detectable labels discussed herein.

[00187] It may be desirable to immobilize either 68730 or 69112, an anti-68730 or anti-69112 antibody or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to a 68730 or 69112 protein, or interaction of a 68730 or 69112 protein with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows

one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase/68730 or glutathione-S-transferase/69112 fusion proteins or glutathione-S-transferase/68730 target or glutathione-S-transferase/69112 fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtiter plates, which are then combined with the test compound or the test compound and either the non-adsorbed 68730 or 69112 target protein or 68730 or 69112 protein, and the mixture incubated under conditions conducive to complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of 68730 or 69112 binding or activity determined using standard techniques.

[00188] Other techniques for immobilizing either a 68730 or 69112 protein or a 68730 or 69112 target molecule on matrices include using conjugation of biotin and streptavidin. Biotinylated 68730 or 69112 protein or 68730 or 69112 target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques known in the art (e.g., biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96-well plates (Pierce Chemical).

[00189] In order to conduct the assay, the non-immobilized component is added to the coated surface containing the anchored component. After the reaction is complete, unreacted components are removed (e.g., by washing) under conditions such that any complexes formed will remain immobilized on the solid surface. The detection of complexes anchored on the solid surface can be accomplished in a number of ways. Where the previously non-immobilized component is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the previously non-immobilized component is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled antibody specific for the immobilized component (the antibody, in turn, can be directly labeled or indirectly labeled with, e.g., a labeled anti-Ig antibody).

[00190] In one embodiment, this assay is performed utilizing antibodies reactive with 68730 or 69112 protein or 68730 or 69112 target molecules but which do not interfere with binding of the 68730 or 69112 protein to its target molecule. Such antibodies can be

derivatized to the wells of the plate, and unbound target or 68730 or 69112 protein trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the 68730 or 69112 protein or target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the 68730 or 69112 protein or 68730 or 69112 target molecule.

[00191] Alternatively, cell free assays can be conducted in a liquid phase. In such an assay, the reaction products are separated from unreacted components, by any of a number of standard techniques, including but not limited to: differential centrifugation (see, for example, Rivas, G., and Minton, A.P., *Trends Biochem Sci* 1993 Aug;18(8):284-7); chromatography (gel filtration chromatography, ion-exchange chromatography); electrophoresis (see, e.g., Ausubel, F. et al., eds. *Current Protocols in Molecular Biology* 1999, J. Wiley: New York.); and immunoprecipitation (see, for example, Ausubel, F. et al., eds. *Current Protocols in Molecular Biology* 1999, J. Wiley: New York). Such resins and chromatographic techniques are known to one skilled in the art (see, e.g., Heegaard, N.H., *J Mol. Recognit.* 1998 Winter;11(1-6):141-8; Hage, D.S., and Tweed, S.A., *J. Chromatogr. B Biomed. Sci. Appl.* 1997 Oct 10;699 (1-2):499-525). Further, fluorescence energy transfer may also be conveniently utilized, as described herein, to detect binding without further purification of the complex from solution.

[00192] In a preferred embodiment, the assay includes contacting the 68730 or 69112 protein, or a biologically active portion thereof, with a known compound which binds 68730 or 69112 to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a 68730 or 69112 protein, wherein determining the ability of the test compound to interact with a 68730 or 69112 protein includes determining the ability of the test compound to preferentially bind to 68730 or 69112, or a biologically active portion thereof, or to modulate the activity of a 68730 or 69112 target molecule, as compared to the known compound.

[00193] The target gene products of the invention can, *in vivo*, interact with one or more cellular or extracellular macromolecules, such as proteins. For the purposes of this discussion, such cellular and extracellular macromolecules are referred to herein as "binding partners." Compounds that disrupt such interactions can be useful in regulating the activity of the target gene product. Such compounds can include, but are not limited

to molecules such as antibodies, peptides, and small molecules. The preferred target genes/products for use in this embodiment are the 68730 and 69112 genes herein identified. In an alternative embodiment, the invention provides methods for determining the ability of the test compound to modulate the activity of a 68730 or 69112 protein through modulation of the activity of a downstream effector of a 68730 or a 69112 target molecule. For example, the activity of the effector molecule on an appropriate target can be determined, or the binding of the effector to an appropriate target can be determined, as previously described.

[00194] To identify compounds that interfere with the interaction between the target gene product and its cellular or extracellular binding partner(s), e.g., a substrate, a reaction mixture containing the target gene product and the binding partner is prepared, under conditions and for a time sufficient, to allow the two products to form complex. In order to test an inhibitory agent, the reaction mixture is provided in the presence and absence of the test compound. The test compound can be initially included in the reaction mixture, or can be added at a time subsequent to the addition of the target gene and its cellular or extracellular binding partner. Control reaction mixtures are incubated without the test compound or with a placebo. The formation of any complexes between the target gene product and the cellular or extracellular binding partner is then detected. The formation of a complex in the control reaction, but not in the reaction mixture containing the test compound, indicates that the compound interferes with the interaction of the target gene product and the interactive binding partner. Additionally, complex formation within reaction mixtures containing the test compound and normal target gene product can also be compared to complex formation within reaction mixtures containing the test compound and mutant target gene product. This comparison can be important in those cases wherein it is desirable to identify compounds that disrupt interactions of mutant but not normal target gene products.

[00195] These assays can be conducted in a heterogeneous or homogeneous format. Heterogeneous assays involve anchoring either the target gene product or the binding partner onto a solid phase, and detecting complexes anchored on the solid phase at the end of the reaction. In homogeneous assays, the entire reaction is carried out in a liquid phase. In either approach, the order of addition of reactants can be varied to obtain different information about the compounds being tested. For example, test compounds that interfere with the interaction between the target gene products and the binding

partners, e.g., by competition, can be identified by conducting the reaction in the presence of the test substance. Alternatively, test compounds that disrupt preformed complexes, e.g., compounds with higher binding constants that displace one of the components from the complex, can be tested by adding the test compound to the reaction mixture after complexes have been formed. The various formats are briefly described below.

[00196] In a heterogeneous assay system, either the target gene product or the interactive cellular or extracellular binding partner, is anchored onto a solid surface (e.g., a microtiter plate), while the non-anchored species is labeled, either directly or indirectly. The anchored species can be immobilized by non-covalent or covalent attachments. Alternatively, an immobilized antibody specific for the species to be anchored can be used to anchor the species to the solid surface.

[00197] In order to conduct the assay, the partner of the immobilized species is exposed to the coated surface with or without the test compound. After the reaction is complete, unreacted components are removed (e.g., by washing) and any complexes formed will remain immobilized on the solid surface. Where the non-immobilized species is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the non-immobilized species is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled antibody specific for the initially non-immobilized species (the antibody, in turn, can be directly labeled or indirectly labeled with, e.g., a labeled anti-Ig antibody). Depending upon the order of addition of reaction components, test compounds that inhibit complex formation or that disrupt preformed complexes can be detected.

[00198] Alternatively, the reaction can be conducted in a liquid phase in the presence or absence of the test compound, the reaction products separated from unreacted components, and complexes detected; e.g., using an immobilized antibody specific for one of the binding components to anchor any complexes formed in solution, and a labeled antibody specific for the other partner to detect anchored complexes. Again, depending upon the order of addition of reactants to the liquid phase, test compounds that inhibit complex or that disrupt preformed complexes can be identified.

[00199] In an alternate embodiment of the invention, a homogeneous assay can be used. For example, a preformed complex of the target gene product and the interactive cellular or extracellular binding partner product is prepared in that either the target gene products or their binding partners are labeled, but the signal generated by the label is

quenched due to complex formation (see, e.g., U.S. Patent No. 4,109,496 that utilizes this approach for immunoassays). The addition of a test substance that competes with and displaces one of the species from the preformed complex will result in the generation of a signal above background. In this way, test substances that disrupt target gene product-binding partner interaction can be identified.

[00200] In yet another aspect, the 68730 and 69112 proteins can be used as "bait proteins" in a two-hybrid assay or three-hybrid assay (see, e.g., U.S. Patent No. 5,283,317; Zervos et al., (1993) *Cell* 72:223-232; Madura et al., (1993) *J. Biol. Chem.* 268:12046-12054; Bartel et al., (1993) *Biotechniques* 14:920-924; Iwabuchi et al., (1993) *Oncogene* 8:1693-1696; and Brent WO94/10300), to identify other proteins, which bind to or interact with 68730 or 69112 ("68730-binding proteins" or "68730-bp", or "69112-binding proteins" or "69112-bp") and are involved in 68730 or 69112 activity, respectively. Such 68730-bps and 69112-bps can be activators or inhibitors of signals by the 68730 or 69112 proteins or 68730 or 69112 targets as, for example, downstream elements of a 68730- or 69112-mediated signaling pathway.

[00201] The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for a 68730 or 69112 protein is fused to a gene encoding the DNA binding domain of a known transcription factor (e.g., GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. (Alternatively, the 68730 or 69112 protein can be the fused to the activator domain.) If the "bait" and the "prey" proteins are able to interact, *in vivo*, forming a 68730- or 69112-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (e.g., LacZ) which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene which encodes the protein which interacts with the 68730 or 69112 protein.

[00202] In another embodiment, modulators of 68730 or 69112 expression are identified. For example, a cell or cell free mixture is contacted with a candidate compound and the expression of 68730 or 69112 mRNA or protein evaluated relative to

the level of expression of 68730 or 69112 mRNA or protein in the absence of the candidate compound. When expression of 68730 or 69112 mRNA or protein is greater in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of 68730 or 69112 mRNA or protein expression. Alternatively, when expression of 68730 or 69112 mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of 68730 or 69112 mRNA or protein expression. The level of 68730 or 69112 mRNA or protein expression can be determined by methods described herein for detecting 68730 or 69112 mRNA or protein.

[00203] In another aspect, the invention pertains to a combination of two or more of the assays described herein. For example, a modulating agent can be identified using a cell-based or a cell free assay, and the ability of the agent to modulate the activity of a 68730 or 69112 protein can be confirmed *in vivo*, e.g., in an animal.

[00204] This invention further pertains to novel agents identified by the above-described screening assays. Accordingly, it is within the scope of this invention to further use an agent identified as described herein (e.g., a 68730 or 69112 modulating agent, an antisense 68730 or 69112 nucleic acid molecule, a 68730- or 69112-specific antibody, or a 68730- or 69112-binding partner) in an appropriate animal model to determine the efficacy, toxicity, side effects, or mechanism of action, of treatment with such an agent. Furthermore, novel agents identified by the above-described screening assays can be used for treatments as described herein.

Detection Assays

[00205] Portions or fragments of the nucleic acid sequences identified herein can be used as polynucleotide reagents. For example, these sequences can be used to: (i) map their respective genes on a chromosome e.g., to locate gene regions associated with genetic disease or to associate 68730 or 69112 with a disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample. These applications are described in the subsections below.

Chromosome Mapping

[00206] The 68730 and 69112 nucleotide sequences, or portions thereof, can be used to map the location of the 68730 and 69112 genes on a chromosome. This process is called

chromosome mapping. Chromosome mapping is useful in correlating the 68730 and 69112 sequences with genes associated with disease.

[00207] Briefly, 68730 and 69112 genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the 68730 and 69112 nucleotide sequences. These primers can then be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the 68730 or 69112 sequences will yield an amplified fragment.

[00208] A panel of somatic cell hybrids in which each cell line contains either a single human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, can allow easy mapping of individual genes to specific human chromosomes. (D'Eustachio P. et al., (1983) *Science* 220:919-924).

[00209] Other mapping strategies e.g., in situ hybridization (described in Fan, Y. et al., (1990) *Proc. Natl. Acad. Sci. USA*, 87:6223-27), pre-screening with labeled flow-sorted chromosomes, and pre-selection by hybridization to chromosome specific cDNA libraries can be used to map 68730 or 69112 to a chromosomal location.

[00210] Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one step. The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. Preferably 1,000 bases, and more preferably 2,000 bases will suffice to get good results at a reasonable amount of time. For a review of this technique, see Verma et al., *Human Chromosomes: A Manual of Basic Techniques* (Pergamon Press, New York 1988).

[00211] Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

[00212] Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. (Such data are found, for example, in V. McKusick, *Mendelian Inheritance in Man*, available on-line through Johns Hopkins University Welch Medical Library). The

relationship between a gene and a disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, for example, Egeland, J. et al., (1987) *Nature*, 325:783-787.

[00213] Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with the 68730 or 69112 gene, can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

Tissue Typing

[00214] 68730 and 69112 sequences can be used to identify individuals from biological samples using, e.g., restriction fragment length polymorphism (RFLP). In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, the fragments separated, e.g., in a Southern blot, and probed to yield bands for identification. The sequences of the present invention are useful as additional DNA markers for RFLP (described in U.S. Patent 5,272,057).

[00215] Furthermore, the sequences of the present invention can also be used to determine the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the 68730 and 69112 nucleotide sequences described herein can be used to prepare two PCR primers from the 5' and 3' ends of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it. Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences.

[00216] Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to

differentiate individuals. The noncoding sequences of SEQ ID NO:1 and 4 can provide positive individual identification with a panel of perhaps 10 to 1,000 primers which each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences, such as those in SEQ ID NO:3 or 5, are used, a more appropriate number of primers for positive individual identification would be 500-2,000.

[00217] If a panel of reagents from 68730 or 69112 nucleotide sequences described herein is used to generate a unique identification database for an individual, those same reagents can later be used to identify tissue from that individual. Using the unique identification database, positive identification of the individual, living or dead, can be made from extremely small tissue samples.

Use of Partial 68730 and 69112 Sequences in Forensic Biology

[00218] DNA-based identification techniques can also be used in forensic biology. To make such an identification, PCR technology can be used to amplify DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, or semen found at a crime scene. The amplified sequence can then be compared to a standard, thereby allowing identification of the origin of the biological sample.

[00219] The sequences of the present invention can be used to provide polynucleotide reagents, e.g., PCR primers, targeted to specific loci in the human genome, which can enhance the reliability of DNA-based forensic identifications by, for example, providing another "identification marker" (i.e. another DNA sequence that is unique to a particular individual). As mentioned above, actual base sequence information can be used for identification as an accurate alternative to patterns formed by restriction enzyme generated fragments. Sequences targeted to noncoding regions of SEQ ID NO:1 or 4 (e.g., fragments derived from the noncoding regions of SEQ ID NO:1 or 4 having a length of at least 20 bases, preferably at least 30 bases) are particularly appropriate for this use.

[00220] The 68730 and 69112 nucleotide sequences described herein can further be used to provide polynucleotide reagents, e.g., labeled or labelable probes which can be used in, for example, an *in situ* hybridization technique, to identify a specific tissue, e.g., a tissue containing protein kinase activity. This can be very useful in cases where a forensic pathologist is presented with a tissue of unknown origin. Panels of such 68730 and 69112 probes can be used to identify tissue by species and/or by organ type.

[00221] In a similar fashion, these reagents, *e.g.*, 68730 and 69112 primers or probes can be used to screen tissue culture for contamination (i.e. screen for the presence of a mixture of different types of cells in a culture).

Predictive Medicine

[00222] The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual.

[00223] Generally, the invention provides, a method of determining if a subject is at risk for a disorder related to a lesion in or the misexpression of a gene which encodes 68730 or 69112. The method includes one or more of the following:

detecting, in a tissue of the subject, the presence or absence of a mutation which affects the expression of the 68730 or 69112 gene, or detecting the presence or absence of a mutation in a region which controls the expression of the gene, *e.g.*, a mutation in the 5' control region;

detecting, in a tissue of the subject, the presence or absence of a mutation which alters the structure of the 68730 or 69112 gene;

detecting, in a tissue of the subject, the misexpression of the 68730 or 69112 gene, at the mRNA level, *e.g.*, detecting a non-wild type level of a mRNA; or

detecting, in a tissue of the subject, the misexpression of the gene, at the protein level, *e.g.*, detecting a non-wild type level of a 68730 or 69112 polypeptide.

[00224] In preferred embodiments the method includes: ascertaining the existence of at least one of: a deletion of one or more nucleotides from the 68730 or 69112 gene; an insertion of one or more nucleotides into the gene, a point mutation, *e.g.*, a substitution of one or more nucleotides of the gene, a gross chromosomal rearrangement of the gene, *e.g.*, a translocation, inversion, or deletion.

[00225] For example, detecting the genetic lesion can include: (i) providing a probe/primer including an oligonucleotide containing a region of nucleotide sequence which hybridizes to a sense or antisense sequence from SEQ ID NO:1 or 4 naturally occurring mutants thereof, or 5' or 3' flanking sequences naturally associated with the 68730 or 69112 gene; (ii) exposing the probe/primer to nucleic acid of the tissue; and detecting, by hybridization, *e.g.*, *in situ* hybridization, of the probe/primer to the nucleic acid, the presence or absence of the genetic lesion.

[00226] In preferred embodiments detecting the misexpression includes ascertaining the existence of at least one of: an alteration in the level of a messenger RNA transcript of the 68730 or 69112 gene; the presence of a non-wild type splicing pattern of a messenger RNA transcript of the gene; or a non-wild type level of 68730 or 69112.

[00227] Methods of the invention can be used prenatally or to determine if a subject's offspring will be at risk for a disorder.

[00228] In preferred embodiments the method includes determining the structure of a 68730 or a 69112 gene, an abnormal structure being indicative of risk for the disorder.

[00229] In preferred embodiments the method includes contacting a sample from the subject with an antibody to the 68730 or 69112 protein or a nucleic acid, which hybridizes specifically with the gene. These and other embodiments are discussed below.

Diagnostic and Prognostic Assays

[00230] The presence, level, or absence of 68730 and 69112 protein or nucleic acid in a biological sample can be evaluated by obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting 68730 or 69112 protein or nucleic acid (e.g., mRNA, genomic DNA) that encodes 68730 or 69112 protein such that the presence of 68730 or 69112 protein or nucleic acid is detected in the biological sample. The term "biological sample" includes tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. A preferred biological sample is serum. The level of expression of the 68730 or 69112 gene can be measured in a number of ways, including, but not limited to: measuring the mRNA encoded by the 68730 or 69112 genes; measuring the amount of protein encoded by the 68730 or 69112 genes; or measuring the activity of the protein encoded by the 68730 or 69112 genes.

[00231] The level of mRNA corresponding to the 68730 or 69112 gene in a cell can be determined both by *in situ* and by *in vitro* formats.

[00232] The isolated mRNA can be used in hybridization or amplification assays that include, but are not limited to, Southern or Northern analyses, polymerase chain reaction analyses and probe arrays. One preferred diagnostic method for the detection of mRNA levels involves contacting the isolated mRNA with a nucleic acid molecule (probe) that can hybridize to the mRNA encoded by the gene being detected. The nucleic acid probe can be, for example, a full-length 68730 or 69112 nucleic acid, such as the nucleic acid of

SEQ ID NO:1, 3, 4, or 6, or a portion thereof, such as an oligonucleotide of at least 7, 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to 68730 or 69112 mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays are described herein.

[00233] In one format, mRNA (or cDNA) is immobilized on a surface and contacted with the probes, for example by running the isolated mRNA on an agarose gel and transferring the mRNA from the gel to a membrane, such as nitrocellulose. In an alternative format, the probes are immobilized on a surface and the mRNA (or cDNA) is contacted with the probes, for example, in a two-dimensional gene chip array. A skilled artisan can adapt known mRNA detection methods for use in detecting the level of mRNA encoded by the 68730 or 69112 genes.

[00234] The level of mRNA in a sample that is transcribed by 68730 or 69112 can be evaluated with nucleic acid amplification, e.g., by rtPCR (Mullis, 1987, U.S. Patent No. 4,683,202), ligase chain reaction (Barany, 1991, *Proc. Natl. Acad. Sci. USA* 88:189-193), self sustained sequence replication (Guatelli et al., 1990, *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh et al., 1989, *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi et al., 1988, *Bio/Technology* 6:1197), rolling circle replication (Lizardi et al., U.S. Patent No. 5,854,033) or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques known in the art. As used herein, amplification primers are defined as being a pair of nucleic acid molecules that can anneal to 5' or 3' regions of a gene (plus and minus strands, respectively, or vice-versa) and contain a short region in between. In general, amplification primers are from about 10 to 30 nucleotides in length and flank a region from about 50 to 200 nucleotides in length. Under appropriate conditions and with appropriate reagents, such primers permit the amplification of a nucleic acid molecule comprising the nucleotide sequence flanked by the primers.

[00235] For *in situ* methods, a cell or tissue sample can be prepared/processed and immobilized on a support, typically a glass slide, and then contacted with a probe that can hybridize to mRNA that encodes the 68730 or 69112 gene being analyzed.

[00236] In another embodiment, the methods further contacting a control sample with a compound or agent capable of detecting 68730 or 69112 mRNA, or genomic DNA, and comparing the presence of 68730 or 69112 mRNA or genomic DNA in the control sample with the presence of 68730 or 69112 mRNA or genomic DNA in the test sample.

[00237] A variety of methods can be used to determine the level of protein encoded by 68730 or 69112. In general, these methods include contacting an agent that selectively binds to the protein, such as an antibody with a sample, to evaluate the level of protein in the sample. In a preferred embodiment, the antibody bears a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (e.g., Fab or F(ab')₂) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (i.e., physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with a detectable substance. Examples of detectable substances are provided herein.

[00238] The detection methods can be used to detect 68730 or 69112 proteins in a biological sample *in vitro* as well as *in vivo*. *In vitro* techniques for detection of 68730 or 69112 protein include enzyme linked immunosorbent assays (ELISAs), immunoprecipitations, immunofluorescence, enzyme immunoassay (EIA), radioimmunoassay (RIA), and Western blot analysis. *In vivo* techniques for detection of 68730 and 69112 proteins include introducing into a subject a labeled anti-68730 or anti-69112 antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

[00239] In another embodiment, the methods further include contacting the control sample with a compound or agent capable of detecting 68730 or 69112 protein, and comparing the presence of 68730 or 69112 protein in the control sample with the presence of 68730 or 69112 protein in the test sample.

[00240] The invention also includes kits for detecting the presence of 68730 or 69112 in a biological sample. For example, the kit can include a compound or agent capable of detecting 68730 or 69112 protein or mRNA in a biological sample; and a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect 68730 or 69112 protein or nucleic acid.

[00241] For antibody-based kits, the kit can include: (1) a first antibody (e.g., attached to a solid support) which binds to a polypeptide corresponding to a marker of the invention; and, optionally, (2) a second, different antibody which binds to either the polypeptide or the first antibody and is conjugated to a detectable agent.

[00242] For oligonucleotide-based kits, the kit can include: (1) an oligonucleotide, e.g., a detectably labeled oligonucleotide, which hybridizes to a nucleic acid sequence

encoding a polypeptide corresponding to a marker of the invention or (2) a pair of primers useful for amplifying a nucleic acid molecule corresponding to a marker of the invention. The kit can also include a buffering agent, a preservative, or a protein-stabilizing agent. The kit can also include components necessary for detecting the detectable agent (e.g., an enzyme or a substrate). The kit can also contain a control sample or a series of control samples which can be assayed and compared to the test sample contained. Each component of the kit can be enclosed within an individual container and all of the various containers can be within a single package, along with instructions for interpreting the results of the assays performed using the kit.

[00243] The diagnostic methods described herein can identify subjects having, or at risk of developing, a disease or disorder associated with misexpressed or aberrant or unwanted 68730 or 69112 expression or activity. As used herein, the term "unwanted" includes an unwanted phenomenon involved in a biological response such as pain or deregulated cell proliferation.

[00244] In one embodiment, a disease or disorder associated with aberrant or unwanted 68730 or 69112 expression or activity is identified. A test sample is obtained from a subject and 68730 or 69112 protein or nucleic acid (e.g., mRNA or genomic DNA) is evaluated, wherein the level, e.g., the presence or absence, of 68730 or 69112 protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant or unwanted 68730 or 69112 expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest, including a biological fluid (e.g., serum), cell sample, or tissue.

[00245] The prognostic assays described herein can be used to determine whether a subject can be administered an agent (e.g., an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant or unwanted 68730 or 69112 expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for a cellular growth related disorder.

[00246] The methods of the invention can also be used to detect genetic alterations in 68730 and 69112 genes, thereby determining if a subject with an altered 68730 or 69112 gene is at risk for a disorder characterized by misregulation in 68730 or 69112 protein activity or nucleic acid expression, such as a cellular growth related disorder. In preferred embodiments, the methods include detecting, in a sample from the subject, the presence

or absence of a genetic alteration characterized by at least one of an alteration affecting the integrity of a gene encoding a 68730 or 69112 protein, or the mis-expression of the 68730 or 69112 gene. For example, such genetic alterations can be detected by ascertaining the existence of at least one of 1) a deletion of one or more nucleotides from a 68730 or 69112 gene; 2) an addition of one or more nucleotides to a 68730 or a 69112 gene; 3) a substitution of one or more nucleotides of a 68730 or 69112 gene, 4) a chromosomal rearrangement of a 68730 or 69112 gene; 5) an alteration in the level of a messenger RNA transcript of a 68730 or 69112 gene, 6) aberrant modification of a 68730 or 69112 gene, such as of the methylation pattern of the genomic DNA, 7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of a 68730 or 69112 gene, 8) a non-wild type level of a 68730 or 69112 protein, 9) allelic loss of a 68730 or a 69112 gene, and 10) inappropriate post-translational modification of a 68730 or a 69112 protein.

[00247] An alteration can be detected without a probe/primer in a polymerase chain reaction, such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR), the latter of which can be particularly useful for detecting point mutations in the 68730 and 69112 genes. This method can include the steps of collecting a sample of cells from a subject, isolating nucleic acid (*e.g.*, genomic, mRNA or both) from the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to a 68730 or a 69112 gene under conditions such that hybridization and amplification of the 68730 or 69112 gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

[00248] Alternative amplification methods include: self sustained sequence replication (Guatelli, J.C. et al., (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh, D.Y. et al., (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi, P.M. et al., (1988) *Bio-Technology* 6:1197), or other nucleic acid amplification methods, followed by the detection of the amplified molecules using techniques known to those of skill in the art.

[00249] In another embodiment, mutations in a 68730 or a 69112 gene from a sample cell can be identified by detecting alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one

or more restriction endonucleases, and fragment length sizes are determined, e.g., by gel electrophoresis, and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (see, for example, U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

[00250] In other embodiments, genetic mutations in 68730 and 69112 can be identified by hybridizing a sample and control nucleic acids, e.g., DNA or RNA, two-dimensional arrays, e.g., chip based arrays. Such arrays include a plurality of addresses, each of which is positionally distinguishable from the other. A different probe is located at each address of the plurality. The arrays can have a high density of addresses, e.g., can contain hundreds or thousands of oligonucleotides probes (Cronin, M.T. et al., (1996) *Human Mutation* 7: 244-255; Kozal, M.J. et al., (1996) *Nature Medicine* 2:753-759). For example, genetic mutations in 68730 or 69112 can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin, M.T. et al., *supra*. Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

[00251] In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the 68730 and 69112 genes and detect mutations by comparing the sequence of the sample 68730 or 69112 with the corresponding wild-type (control) sequence. Automated sequencing procedures can be utilized when performing the diagnostic assays ((1995) *Biotechniques* 19:448), including sequencing by mass spectrometry.

[00252] Other methods for detecting mutations in the 68730 and 69112 genes include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers et al., (1985) *Science* 230:1242; Cotton et al., (1988) *Proc. Natl. Acad. Sci. USA* 85:4397; Saleeba et al., (1992) *Methods Enzymol.* 217:286-295).

[00253] In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in 68730 or 69112 cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu et al., (1994) *Carcinogenesis* 15:1657-1662; U.S. Patent No. 5,459,039).

[00254] In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in 68730 and 69112 genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita et al., (1989) *Proc. Natl. Acad. Sci. USA*: 86:2766, see also Cotton, (1993) *Mutat. Res.* 285:125-144; and Hayashi, (1992) *Genet. Anal. Tech. Appl.* 9:73-79). Single-stranded DNA fragments of sample and control 68730 or 69112 nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In a preferred embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen et al., (1991) *Trends Genet.* 7:5).

[00255] In yet another embodiment, the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers et al., (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner, (1987) *Biophys. Chem.* 265:12753).

[00256] Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective

primer extension (Saiki et al., (1986) *Nature* 324:163); Saiki et al., (1989) *Proc. Natl. Acad. Sci. USA* 86:6230).

[00257] Alternatively, allele specific amplification technology which depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs et al., (1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner, (1993) *Tibtech* 11:238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini et al., (1992) *Mol. Cell Probes* 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany, (1991) *Proc. Natl. Acad. Sci. USA* 88:189). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

[00258] The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, *e.g.*, in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving a 68730 or a 69112 gene.

Use of 68730 and 69112 Molecules as Surrogate Markers

[00259] The 68730 and 69112 molecules of the invention are also useful as markers of disorders or disease states, as markers for precursors of disease states, as markers for predisposition of disease states, as markers of drug activity, or as markers of the pharmacogenomic profile of a subject. Using the methods described herein, the presence, absence and/or quantity of the 68730 and 69112 molecules of the invention may be detected, and may be correlated with one or more biological states *in vivo*. For example, the 68730 or 69112 molecules of the invention may serve as surrogate markers for one or more disorders or disease states or for conditions leading up to disease states. As used herein, a "surrogate marker" is an objective biochemical marker which correlates with the absence or presence of a disease or disorder, or with the progression of a disease or

disorder (*e.g.*, with the presence or absence of a tumor). The presence or quantity of such markers is independent of the disease. Therefore, these markers may serve to indicate whether a particular course of treatment is effective in lessening a disease state or disorder. Surrogate markers are of particular use when the presence or extent of a disease state or disorder is difficult to assess through standard methodologies (*e.g.*, early stage tumors), or when an assessment of disease progression is desired before a potentially dangerous clinical endpoint is reached (*e.g.*, an assessment of cardiovascular disease may be made using cholesterol levels as a surrogate marker, and an analysis of HIV infection may be made using HIV RNA levels as a surrogate marker, well in advance of the undesirable clinical outcomes of myocardial infarction or fully-developed AIDS). Examples of the use of surrogate markers in the art include: Koomen *et al.* (2000) *J. Mass. Spectrom.* 35: 258-264; and James (1994) *AIDS Treatment News Archive* 209.

[00260] The 68730 and 69112 molecules of the invention are also useful as pharmacodynamic markers. As used herein, a “pharmacodynamic marker” is an objective biochemical marker which correlates specifically with drug effects. The presence or quantity of a pharmacodynamic marker is not related to the disease state or disorder for which the drug is being administered; therefore, the presence or quantity of the marker is indicative of the presence or activity of the drug in a subject. For example, a pharmacodynamic marker may be indicative of the concentration of the drug in a biological tissue, in that the marker is either expressed or transcribed or not expressed or transcribed in that tissue in relationship to the level of the drug. In this fashion, the distribution or uptake of the drug may be monitored by the pharmacodynamic marker. Similarly, the presence or quantity of the pharmacodynamic marker may be related to the presence or quantity of the metabolic product of a drug, such that the presence or quantity of the marker is indicative of the relative breakdown rate of the drug *in vivo*.

Pharmacodynamic markers are of particular use in increasing the sensitivity of detection of drug effects, particularly when the drug is administered in low doses. Since even a small amount of a drug may be sufficient to activate multiple rounds of marker (*e.g.*, a 68730 or a 69112 marker) transcription or expression, the amplified marker may be in a quantity which is more readily detectable than the drug itself. Also, the marker may be more easily detected due to the nature of the marker itself; for example, using the methods described herein, anti-68730 and anti-69112 antibodies may be employed in an immune-based detection system for a 68730 or a 69112 protein marker, or 68730- or

69112-specific radiolabeled probes may be used to detect a 68730 or 69112 mRNA marker. Furthermore, the use of a pharmacodynamic marker may offer mechanism-based prediction of risk due to drug treatment beyond the range of possible direct observations. Examples of the use of pharmacodynamic markers in the art include: Matsuda *et al.* US 6,033,862; Hattis *et al.* (1991) *Env. Health Perspect.* 90: 229-238; Schentag (1999) *Am. J. Health-Syst. Pharm.* 56 Suppl. 3: S21-S24; and Nicolau (1999) *Am. J. Health-Syst. Pharm.* 56 Suppl. 3: S16-S20.

[00261] The 68730 and 69112 molecules of the invention are also useful as pharmacogenomic markers. As used herein, a "pharmacogenomic marker" is an objective biochemical marker which correlates with a specific clinical drug response or susceptibility in a subject (see, e.g., McLeod *et al.* (1999) *Eur. J. Cancer* 35(12): 1650-1652). The presence or quantity of the pharmacogenomic marker is related to the predicted response of the subject to a specific drug or class of drugs prior to administration of the drug. By assessing the presence or quantity of one or more pharmacogenomic markers in a subject, a drug therapy which is most appropriate for the subject, or which is predicted to have a greater degree of success, may be selected. For example, based on the presence or quantity of RNA, or protein (e.g., 68730 or 69112 protein or RNA) for specific tumor markers in a subject, a drug or course of treatment may be selected that is optimized for the treatment of the specific tumor likely to be present in the subject. Similarly, the presence or absence of a specific sequence mutation in 68730 or 69112 DNA may correlate 68730 or 69112 drug response. The use of pharmacogenomic markers therefore permits the application of the most appropriate treatment for each subject without having to administer the therapy.

Pharmaceutical Compositions

[00262] The nucleic acid and polypeptides, fragments thereof, as well as anti-68730 and anti-69112 antibodies, and modulators of 68730 or 69112 expression or activity identified in screening assays described above (e.g., peptidomimetics, peptoids, and small molecules) (also referred to herein as "active compounds") of the invention can be incorporated into pharmaceutical compositions. Such compositions typically include the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" includes solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption

delaying agents, and the like, compatible with pharmaceutical administration.

Supplementary active compounds can also be incorporated into the compositions.

[00263] A pharmaceutical composition is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[00264] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It should be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about

by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

[00265] Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[00266] Oral compositions generally include an inert diluent or an edible carrier. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules, e.g., gelatin capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

[00267] For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

[00268] Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active

compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

[00269] The compounds can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

[00270] In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems.

Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid.

Methods for preparation of such formulations will be apparent to those skilled in the art.

The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

[00271] It is advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

[00272] Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD₅₀/ED₅₀. Compounds which exhibit high therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

[00273] The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies

preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

[00274] As defined herein, a therapeutically effective amount of protein or polypeptide (*i.e.*, an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about 0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight. The protein or polypeptide can be administered one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. The skilled artisan will appreciate that certain factors may influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a protein, polypeptide, or antibody can include a single treatment or, preferably, can include a series of treatments.

[00275] For antibodies, the preferred dosage is 0.1 mg/kg of body weight (generally 10 mg/kg to 20 mg/kg). If the antibody is to act in the brain, a dosage of 50 mg/kg to 100 mg/kg is usually appropriate. Generally, partially human antibodies and fully human antibodies have a longer half-life within the human body than other antibodies. Accordingly, lower dosages and less frequent administration is often possible. Modifications such as lipidation can be used to stabilize antibodies and to enhance uptake and tissue penetration (*e.g.*, into the brain). A method for lipidation of antibodies is described by Cruikshank et al., ((1997) *J. Acquired Immune Deficiency Syndromes and Human Retrovirology* 14:193).

[00276] The present invention encompasses agents which modulate expression or activity. An agent may, for example, be a small molecule. For example, such small molecules include, but are not limited to, peptides, peptidomimetics (e.g., peptoids), amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (i.e., including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds.

[00277] Exemplary doses include milligram or microgram amounts of the small molecule per kilogram of subject or sample weight (e.g., about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram. It is furthermore understood that appropriate doses of a small molecule depend upon the potency of the small molecule with respect to the expression or activity to be modulated. When one or more of these small molecules is to be administered to an animal (e.g., a human) in order to modulate expression or activity of a polypeptide or nucleic acid of the invention, a physician, veterinarian, or researcher may, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. In addition, it is understood that the specific dose level for any particular animal subject will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or activity to be modulated.

[00278] An antibody (or fragment thereof) may be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent or a radioactive metal ion. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotosterone,

glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

[00279] The conjugates of the invention can be used for modifying a given biological response, the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, .alpha.-interferon, .beta.-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

[00280] Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980.

[00281] The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by stereotactic injection (see e.g., Chen et al., (1994) *Proc. Natl. Acad. Sci. USA* 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, e.g., retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

[00282] The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

Methods of Treatment

[00283] The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant or unwanted 68730 or 69112 expression or activity. With regard to both prophylactic and therapeutic methods of treatment, such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics. "Pharmacogenomics", as used herein, refers to the application of genomics technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. More specifically, the term refers the study of how a patient's genes determine his or her response to a drug (e.g., a patient's "drug response phenotype", or "drug response genotype".) Thus, another aspect of the invention provides methods for tailoring an individual's prophylactic or therapeutic treatment with either the 68730 or 69112 molecules of the present invention or 68730 or 69112 modulators according to that individual's drug response genotype.

Pharmacogenomics allows a clinician or physician to target prophylactic or therapeutic treatments to patients who will most benefit from the treatment and to avoid treatment of patients who will experience toxic drug-related side effects.

[00284] "Treatment", as used herein, is defined as the application or administration of a therapeutic agent to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient, who has a disease, a symptom of disease or a predisposition toward a disease, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, palliate, improve or affect the disease, the symptoms of disease or the predisposition toward disease. A therapeutic agent includes, but is not limited to, small molecules, peptides, antibodies, ribozymes and antisense oligonucleotides.

[00285] In one aspect, the invention provides a method for preventing in a subject, a disease or condition associated with an aberrant or unwanted 68730 or 69112 expression or activity, by administering to the subject a 68730 or 69112 or an agent which modulates 68730 or 69112 expression or at least one 68730 or 69112 activity. Subjects at risk for a disease which is caused or contributed to by aberrant or unwanted 68730 or 69112 expression or activity can be identified by, for example, any or a combination of

diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the 68730 or 69112 aberrance, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of 68730 or 69112 aberrance, for example, a 68730 or 69112 agonist or 68730 or 69112 antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein.

[00286] It is possible that some 68730 or 69112 disorders can be caused, at least in part, by an abnormal level of gene product, or by the presence of a gene product exhibiting abnormal activity. As such, the reduction in the level and/or activity of such gene products would bring about the amelioration of disorder symptoms.

[00287] As discussed, successful treatment of 68730 or 69112 disorders can be brought about by techniques that serve to inhibit the expression or activity of 68730 or 69112 target gene products. For example, compounds, e.g., an agent identified using an assays described above, that proves to exhibit negative modulatory activity, can be used in accordance with the invention to prevent and/or ameliorate symptoms of 68730 or 69112 disorders. Such molecules can include, but are not limited to peptides, phosphopeptides, small organic or inorganic molecules, or antibodies (including, for example, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain antibodies, and Fab, F(ab')₂ and FAb expression library fragments, scFV molecules, and epitope-binding fragments thereof).

[00288] Further, antisense and ribozyme molecules that inhibit expression of the target gene can also be used in accordance with the invention to reduce the level of target gene expression, thus effectively reducing the level of target gene activity. Still further, triple helix molecules can be utilized in reducing the level of target gene activity. Antisense, ribozyme and triple helix molecules are discussed above.

[00289] It is possible that the use of antisense, ribozyme, and/or triple helix molecules to reduce or inhibit mutant gene expression can also reduce or inhibit the transcription (triple helix) and/or translation (antisense, ribozyme) of mRNA produced by normal target gene alleles, such that the concentration of normal target gene product present can be lower than is necessary for a normal phenotype. In such cases, nucleic acid molecules that encode and express target gene polypeptides exhibiting normal target gene activity can be introduced into cells via gene therapy method. Alternatively, in instances in that

the target gene encodes an extracellular protein, it can be preferable to co-administer normal target gene protein into the cell or tissue in order to maintain the requisite level of cellular or tissue target gene activity.

[00290] Another method by which nucleic acid molecules may be utilized in treating or preventing a disease characterized by 68730 or 69112 expression is through the use of aptamer molecules specific for 68730 or 69112 protein. Aptamers are nucleic acid molecules having a tertiary structure which permits them to specifically bind to protein ligands (see, e.g., Osborne, et al., *Curr. Opin. Chem. Biol.* 1997, 1(1): 5-9; and Patel, D.J., *Curr. Opin. Chem. Biol.* 1997 Jun;1(1):32-46). Since nucleic acid molecules may in many cases be more conveniently introduced into target cells than therapeutic protein molecules may be, aptamers offer a method by which 68730 or 69112 protein activity may be specifically decreased without the introduction of drugs or other molecules which may have pluripotent effects.

[00291] Antibodies can be generated that are both specific for target gene product and that reduce target gene product activity. Such antibodies may, therefore, be administered in instances whereby negative modulatory techniques are appropriate for the treatment of 68730 or 69112 disorders. For a description of antibodies, see the Antibody section above.

[00292] In circumstances wherein injection of an animal or a human subject with a 68730 or 69112 protein or epitope for stimulating antibody production is harmful to the subject, it is possible to generate an immune response against 68730 or 69112 through the use of anti-idiotypic antibodies (see, for example, Herlyn, D., *Ann. Med.* 1999;31(1):66-78; and Bhattacharya-Chatterjee, M., and Foon, K.A., *Cancer Treat. Res.* 1998;94:51-68). If an anti-idiotypic antibody is introduced into a mammal or human subject, it should stimulate the production of anti-anti-idiotypic antibodies, which should be specific to the 68730 or 69112 protein. Vaccines directed to a disease characterized by 68730 or 69112 expression may also be generated in this fashion.

[00293] In instances where the target antigen is intracellular and whole antibodies are used, internalizing antibodies may be preferred. Lipofectin or liposomes can be used to deliver the antibody or a fragment of the Fab region that binds to the target antigen into cells. Where fragments of the antibody are used, the smallest inhibitory fragment that binds to the target antigen is preferred. For example, peptides having an amino acid sequence corresponding to the Fv region of the antibody can be used. Alternatively,

single chain neutralizing antibodies that bind to intracellular target antigens can also be administered. Such single chain antibodies can be administered, for example, by expressing nucleotide sequences encoding single-chain antibodies within the target cell population (see e.g., Marasco et al., (1993, *Proc. Natl. Acad. Sci. USA* 90:7889-7893).

[00294] The identified compounds that inhibit target gene expression, synthesis and/or activity can be administered to a patient at therapeutically effective doses to prevent, treat or ameliorate 68730 or 69112 disorders. A therapeutically effective dose refers to that amount of the compound sufficient to result in amelioration of symptoms of the disorders.

[00295] Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD₅₀/ED₅₀. Compounds that exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects can be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

[00296] The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (i.e., the concentration of the test compound that achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma can be measured, for example, by high performance liquid chromatography.

[00297] Another example of determination of effective dose for an individual is the ability to directly assay levels of "free" and "bound" compound in the serum of the test subject. Such assays may utilize antibody mimics and/or "biosensors" that have been created through molecular imprinting techniques. The compound which is able to

modulate 68730 or 69112 activity is used as a template, or “imprinting molecule”, to spatially organize polymerizable monomers prior to their polymerization with catalytic reagents. The subsequent removal of the imprinted molecule leaves a polymer matrix which contains a repeated “negative image” of the compound and is able to selectively rebind the molecule under biological assay conditions. A detailed review of this technique can be seen in Ansell, R. J. et al., (1996) *Current Opinion in Biotechnology* 7:89-94 and in Shea, K.J., (1994) *Trends in Polymer Science* 2:166-173. Such “imprinted” affinity matrixes are amenable to ligand-binding assays, whereby the immobilized monoclonal antibody component is replaced by an appropriately imprinted matrix. An example of the use of such matrixes in this way can be seen in Vlatakis, G. et al., (1993) *Nature* 361:645-647. Through the use of isotope-labeling, the “free” concentration of compound which modulates the expression or activity of 68730 or 69112 can be readily monitored and used in calculations of IC₅₀.

[00298] Such “imprinted” affinity matrixes can also be designed to include fluorescent groups whose photon-emitting properties measurably change upon local and selective binding of target compound. These changes can be readily assayed in real time using appropriate fiberoptic devices, in turn allowing the dose in a test subject to be quickly optimized based on its individual IC₅₀. A rudimentary example of such a “biosensor” is discussed in Kriz, D. et al., (1995) *Analytical Chemistry* 67:2142-2144.

[00299] Another aspect of the invention pertains to methods of modulating 68730 and 69112 expression or activity for therapeutic purposes. Accordingly, in an exemplary embodiment, the modulatory method of the invention involves contacting a cell with 68730 or 69112 or agent that modulates one or more of the activities of 68730 or 69112 protein activity associated with the cell. An agent that modulates 68730 or 69112 protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring target molecule of a 68730 or 69112 protein (*e.g.*, a 68730 or 69112 substrate or receptor), a anti-68730 or anti-69112 antibody, a 68730 or 69112 agonist or antagonist, a peptidomimetic of a 68730 or 69112 agonist or antagonist, or other small molecule.

[00300] In one embodiment, the agent stimulates one or 68730 or 69112 activities. Examples of such stimulatory agents include active 68730 and 69112 proteins and nucleic acid molecules encoding 68730 or 69112. In another embodiment, the agent inhibits one or more 68730 or 69112 activities. Examples of such inhibitory agents include antisense

68730 and 69112 nucleic acid molecules, anti-68730 and anti-69112 antibodies, and 68730 and 69112 inhibitors. These modulatory methods can be performed *in vitro* (e.g., by culturing the cell with the agent) or, alternatively, *in vivo* (e.g., by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant or unwanted expression or activity of a 68730 or a 69112 protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (e.g., an agent identified by a screening assay described herein), or combination of agents that modulates (e.g., upregulates or downregulates) 68730 or 69112 expression or activity. In another embodiment, the method involves administering a 68730 or 69112 protein or nucleic acid molecule as therapy to compensate for reduced, aberrant, or unwanted 68730 or 69112b expression or activity.

[00301] Stimulation of 68730 or 69112 activity is desirable in situations in which 68730 or 69112 is abnormally downregulated and/or in which increased 68730 or 69112 activity is likely to have a beneficial effect. For example, stimulation of 68730 or 69112 activity is desirable in situations in which a 68730 or a 69112 is downregulated and/or in which increased 68730 or 69112 activity is likely to have a beneficial effect. Likewise, inhibition of 68730 or 69112 activity is desirable in situations in which 68730 or 69112 is abnormally upregulated and/or in which decreased 68730 or 69112 activity is likely to have a beneficial effect.

[00302] The 68730 and 69112 molecules can act as novel diagnostic targets and therapeutic agents for controlling one or more of cellular proliferative and/or differentiative disorders, cardiovascular disorders, as described above, as well as disorders associated with bone metabolism, hematopoietic disorders, liver disorders, viral diseases, pain or metabolic disorders.

[00303] Aberrant expression and/or activity of 68730 or 69112 molecules may mediate disorders associated with bone metabolism. "Bone metabolism" refers to direct or indirect effects in the formation or degeneration of bone structures, e.g., bone formation, bone resorption, etc., which may ultimately affect the concentrations in serum of calcium and phosphate. This term also includes activities mediated by 68730 or 69112 molecules effects in bone cells, e.g. osteoclasts and osteoblasts, that may in turn result in bone formation and degeneration. For example, 68730 or 69112 molecules may support different activities of bone resorbing osteoclasts such as the stimulation of differentiation

of monocytes and mononuclear phagocytes into osteoclasts. Accordingly, 68730 or 69112 molecules that modulate the production of bone cells can influence bone formation and degeneration, and thus may be used to treat bone disorders. Examples of such disorders include, but are not limited to, osteoporosis, osteodystrophy, osteomalacia, rickets, osteitis fibrosa cystica, renal osteodystrophy, osteosclerosis, anti-convulsant treatment, osteopenia, fibrogenesis-imperfecta ossium, secondary hyperparathyroidism, hypoparathyroidism, hyperparathyroidism, cirrhosis, obstructive jaundice, drug induced metabolism, medullary carcinoma, chronic renal disease, rickets, sarcoidosis, glucocorticoid antagonism, malabsorption syndrome, steatorrhea, tropical sprue, idiopathic hypercalcemia and milk fever.

[00304] Disorders which can be treated or diagnosed by methods described herein include, but are not limited to, disorders associated with an accumulation in the liver of fibrous tissue, such as that resulting from an imbalance between production and degradation of the extracellular matrix accompanied by the collapse and condensation of preexisting fibers. The methods described herein can be used to diagnose or treat hepatocellular necrosis or injury induced by a wide variety of agents including processes which disturb homeostasis, such as an inflammatory process, tissue damage resulting from toxic injury or altered hepatic blood flow, and infections (e.g., bacterial, viral and parasitic). For example, the methods can be used for the early detection of hepatic injury, such as portal hypertension or hepatic fibrosis. In addition, the methods can be employed to detect liver fibrosis attributed to inborn errors of metabolism, for example, fibrosis resulting from a storage disorder such as Gaucher's disease (lipid abnormalities) or a glycogen storage disease, A1-antitrypsin deficiency; a disorder mediating the accumulation (e.g., storage) of an exogenous substance, for example, hemochromatosis (iron-overload syndrome) and copper storage diseases (Wilson's disease), disorders resulting in the accumulation of a toxic metabolite (e.g., tyrosinemia, fructosemia and galactosemia) and peroxisomal disorders (e.g., Zellweger syndrome). Additionally, the methods described herein may be useful for the early detection and treatment of liver injury associated with the administration of various chemicals or drugs, such as for example, methotrexate, isoniazid, oxyphenisatin, methyl dopa, chlorpromazine, tolbutamide or alcohol, or which represents a hepatic manifestation of a vascular disorder such as obstruction of either the intrahepatic or extrahepatic bile flow or an alteration in

hepatic circulation resulting, for example, from chronic heart failure, veno-occlusive disease, portal vein thrombosis or Budd-Chiari syndrome.

[00305] Additionally, 68730 and 69112 molecules may play an important role in the etiology of certain viral diseases, including but not limited to, Hepatitis B, Hepatitis C and Herpes Simplex Virus (HSV). Modulators of 68730 or 69112 activity can be used to control viral diseases. The modulators can be used in the treatment and/or diagnosis of viral infected tissue or virus-associated tissue fibrosis, especially liver and liver fibrosis. Also, 68730 and 69112 modulators can be used in the treatment and/or diagnosis of virus-associated carcinomas, especially hepatocellular cancers.

[00306] Additionally, 68730 or 69112 may play an important role in the regulation of metabolism or pain disorders. Diseases of metabolic imbalance include, but are not limited to, obesity, anorexia nervosa, bulimia, cachexia, lipid disorders, and diabetes. Examples of pain disorders include, but are not limited to, pain response elicited during various forms of tissue injury, e.g., inflammation, infection, and ischemia, usually referred to as hyperalgesia (described in, for example, Fields, H.L., (1987) *Pain*, New York:McGraw-Hill); pain associated with musculoskeletal disorders, e.g., joint pain; tooth pain; headaches; pain associated with surgery; pain related to irritable bowel syndrome; or chest pain.

Pharmacogenomics

[00307] The 68730 and 69112 molecules of the present invention, as well as agents, or modulators which have a stimulatory or inhibitory effect on 68730 or 69112 activity (e.g., 68730 or 69112 gene expression) as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) 68730 or 69112 associated disorders (e.g., cellular growth related disorders) associated with aberrant or unwanted 68730 or 69112 activity. In conjunction with such treatment, pharmacogenomics (i.e., the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, a physician or clinician may consider applying knowledge obtained in relevant pharmacogenomics studies in determining whether to administer a 68730 or a 69112 molecule or a 68730 or a 69112 modulator as well as

tailoring the dosage and/or therapeutic regimen of treatment with a 68730 or 69112 molecule or modulator.

[00308] Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See, for example, Eichelbaum, M. et al. (1996) *Clin. Exp. Pharmacol. Physiol.* 23(10-11) :983-985 and Linder, M.W. et al. (1997) *Clin. Chem.* 43(2):254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare genetic defects or as naturally-occurring polymorphisms. For example, glucose-6-phosphate dehydrogenase deficiency (G6PD) is a common inherited enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

[00309] One pharmacogenomics approach to identifying genes that predict drug response, known as "a genome-wide association", relies primarily on a high-resolution map of the human genome consisting of already known gene-related markers (e.g., a "bi-allelic" gene marker map which consists of 60,000-100,000 polymorphic or variable sites on the human genome, each of which has two variants.) Such a high-resolution genetic map can be compared to a map of the genome of each of a statistically significant number of patients taking part in a Phase II/III drug trial to identify markers associated with a particular observed drug response or side effect. Alternatively, such a high-resolution map can be generated from a combination of some ten million known single nucleotide polymorphisms (SNPs) in the human genome. As used herein, a "SNP" is a common alteration that occurs in a single nucleotide base in a stretch of DNA. For example, a SNP may occur once per every 1000 bases of DNA. A SNP may be involved in a disease process, however, the vast majority may not be disease-associated. Given a genetic map based on the occurrence of such SNPs, individuals can be grouped into genetic categories depending on a particular pattern of SNPs in their individual genome. In such a manner, treatment regimens can be tailored to groups of genetically similar individuals, taking into account traits that may be common among such genetically similar individuals.

[00310] Alternatively, a method termed the "candidate gene approach", can be utilized to identify genes that predict drug response. According to this method, if a gene that encodes a drug's target is known (e.g., a 68730 or a 69112 protein of the present invention), all common variants of that gene can be fairly easily identified in the population and it can be determined if having one version of the gene versus another is associated with a particular drug response.

[00311] Alternatively, a method termed the "gene expression profiling", can be utilized to identify genes that predict drug response. For example, the gene expression of an animal dosed with a drug (e.g., a 68730 or 69112 molecule or 68730 or 69112 modulator of the present invention) can give an indication whether gene pathways related to toxicity have been turned on.

[00312] Information generated from more than one of the above pharmacogenomics approaches can be used to determine appropriate dosage and treatment regimens for prophylactic or therapeutic treatment of an individual. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with a 68730 or 69112 molecule or 68730 or 69112 modulator, such as a modulator identified by one of the exemplary screening assays described herein.

[00313] The present invention further provides methods for identifying new agents, or combinations, that are based on identifying agents that modulate the activity of one or more of the gene products encoded by one or more of the 68730 or 69112 genes of the present invention, wherein these products may be associated with resistance of the cells to a therapeutic agent. Specifically, the activity of the proteins encoded by the 68730 or 69112 genes of the present invention can be used as a basis for identifying agents for overcoming agent resistance. By blocking the activity of one or more of the resistance proteins, target cells, e.g., cancer cells, will become sensitive to treatment with an agent that the unmodified target cells were resistant to.

[00314] Monitoring the influence of agents (e.g., drugs) on the expression or activity of a 68730 or 69112 protein can be applied in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase 68730 or 69112 gene expression, protein levels, or upregulate 68730 or 69112 activity, can be monitored in clinical trials of subjects exhibiting decreased 68730 or 69112 gene expression, protein levels, or downregulated 68730 or 69112 activity. Alternatively, the

effectiveness of an agent determined by a screening assay to decrease 68730 or 69112 gene expression, protein levels, or downregulate 68730 or 69112 activity, can be monitored in clinical trials of subjects exhibiting increased 68730 or 69112 gene expression, protein levels, or upregulated 68730 or 69112 activity. In such clinical trials, the expression or activity of a 68730 or 69112 gene, and preferably, other genes that have been implicated in, for example, a 68730-or 69112-associated disorder can be used as a "read out" or markers of the phenotype of a particular cell.

Other Embodiments

[00315] In another aspect, the invention features, a method of analyzing a plurality of capture probes. The method can be used, e.g., to analyze gene expression. The method includes: providing a two dimensional array having a plurality of addresses, wherein each address of the plurality is positionally distinguishable from every other address of the plurality, and each address of the plurality has a unique capture probe, e.g., a nucleic acid or peptide sequence; contacting the array with a 68730 or 69112 molecule, e.g., a 68730 or 69112 nucleic acid, preferably purified, a 68730 or 69112 polypeptide, preferably purified, or an anti-68730 or anti-69112 antibody, preferably purified, and evaluating the plurality of capture probes. Binding with a capture probe at an address of the plurality, is detected, e.g., by a signal generated from a label attached to the 68730 or 69112 nucleic acid, polypeptide, or antibody.

[00316] The capture probes can be a set of nucleic acids from a selected sample, e.g., a sample of nucleic acids derived from a control or non-stimulated tissue or cell.

[00317] The method can include contacting the 68730 or 69112 nucleic acid, polypeptide, or antibody with a first array having a plurality of capture probes and a second array having a different plurality of capture probes. The results of each hybridization can be compared, e.g., to analyze differences in expression between a first and second sample. The first plurality of capture probes can be from a control sample, e.g., a wild type, normal, or non-diseased, non-stimulated, sample, e.g., a biological fluid, tissue, or cell sample. The second plurality of capture probes can be from an experimental sample, e.g., a mutant type, at risk, disease-state or disorder-state, or stimulated, sample, e.g., a biological fluid, tissue, or cell sample.

[00318] The plurality of capture probes can be a plurality of nucleic acid probes each of which specifically hybridizes, with an allele of 68730 or 69112. Such methods can be

used to diagnose a subject, e.g., to evaluate risk for a disease or disorder, to evaluate suitability of a selected treatment for a subject, to evaluate whether a subject has a disease or disorder.

[00319] The method can be used to detect SNPs, as described above.

[00320] In another aspect, the invention features, a method of analyzing a plurality of probes. The method is useful, e.g., for analyzing gene expression. The method includes: providing a two dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality having a unique capture probe, e.g., wherein the capture probes are from a cell or subject which expresses or misexpresses 68730 or 69112 or from a cell or subject in which a 68730 or 69112 mediated response has been elicited, e.g., by contact of the cell with 68730 or 69112 nucleic acid or protein, or administration to the cell or subject 68730 or 69112 nucleic acid or protein; contacting the array with one or more inquiry probe, wherein an inquiry probe can be a nucleic acid, polypeptide, or antibody (which is preferably other than 68730 or 69112 nucleic acid, polypeptide, or antibody); providing a two dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality, and each address of the plurality having a unique capture probe, e.g., wherein the capture probes are from a cell or subject which does not express 68730 or 69112 (or does not express as highly as in the case of the 68730 or 69112 positive plurality of capture probes) or from a cell or subject which in which a 68730- or 69112-mediated response has not been elicited (or has been elicited to a lesser extent than in the first sample); contacting the array with one or more inquiry probes (which is preferably other than a 68730 or 69112 nucleic acid, polypeptide, or antibody), and thereby evaluating the plurality of capture probes. Binding, e.g., in the case of a nucleic acid, hybridization with a capture probe at an address of the plurality, is detected, e.g., by signal generated from a label attached to the nucleic acid, polypeptide, or antibody.

[00321] In another aspect, the invention features, a method of analyzing 68730 or 69112, e.g., analyzing structure, function, or relatedness to other nucleic acid or amino acid sequences. The method includes: providing a 68730 or 69112 nucleic acid or amino acid sequence; comparing the 68730 or 69112 sequence with one or more preferably a plurality of sequences from a collection of sequences, e.g., a nucleic acid or protein sequence database; to thereby analyze 68730 or 69112.

[00322] Preferred databases include GenBank™. The method can include evaluating the sequence identity between a 68730 or 69112 sequence and a database sequence. The method can be performed by accessing the database at a second site, e.g., over the internet.

[00323] In another aspect, the invention features, a set of oligonucleotides, useful, e.g., for identifying SNP's, or identifying specific alleles of 68730 or 69112. The set includes a plurality of oligonucleotides, each of which has a different nucleotide at an interrogation position, e.g., an SNP or the site of a mutation. In a preferred embodiment, the oligonucleotides of the plurality identical in sequence with one another (except for differences in length). The oligonucleotides can be provided with different labels, such that an oligonucleotides which hybridizes to one allele provides a signal that is distinguishable from an oligonucleotides which hybridizes to a second allele.

[00324] This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application are incorporated herein by reference.

Equivalents

[00325] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.